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THE BREEDING OF IMPROVED VARIETIES OF SPRING WHEAT[1]

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During the last twenty or twenty-five years there has been a great change in the type of agriculture practiced in Minnesota. Diversification of crops has made material progress in practically all sections of the state and has replaced the one-type farming system exemplified by the growing of wheat continuously. Corn has superseded wheat as the most important crop grown in the state, approximately 25 per cent of the crop area being planted to corn; and the acreage of spring wheat has been reduced from more than 4 million acres in 1912 to 1,653,000 in 1927. The acreage of winter wheat has increased from 50,000 acres in 1913 to 155,000 acres in 1927. This increase was made possible largely by the distribution of Minturki, a winter-hardy variety that appears especially well adapted to southern Minnesota. The increased acreage is an indication that wheat is still a profitable cash crop for Minnesota farmers where it can be grown successfully.

Some crops are affected by seasonal conditions to a much greater extent than others. Other things being equal, there is an advantage in growing the crop that has the lowest seasonal variation in yield. The comparative seasonal variability in yield of corn, spring wheat, oats, and barley in Minnesota can be determined by a study of variations from year to year. Statistics for these four crops for the last twenty years have been used to emphasize seasonal variability, the coefficient of variability being the standard of comparison.

Crop	Range in yield per acre 1908-27 bu.	Coefficient of variability
Corn	23.0-41.0	13.46 ± 1.46
Barley	19.0-32.0	17.12 ± 1.89
Oats	22.0-43.0	21.59 ± 2.41
Spring wheat	7.5-21.8	28.29 ± 3.25

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The question naturally arises: Is seasonal variability due to something inherent in the spring wheat crop that it is impossible to regulate, or is it the result of factors, environmental or hereditary, that can be controlled? Many data lead to the conclusion that plant diseases are the chief cause for the extreme variability in the yield of spring wheat as compared with that of some other crops.

Stem rust was epidemic in the spring wheat area in 1916, 1919, and 1920. In these three years the average yield was 8.6 bushels per acre. In the six other years from 1912 to 1920, when stem rust was not so severe, the average yield was 15.4 bushels.

The only stem-rust spores that can overwinter in northern latitudes are the teleospores, which are formed on the host plant in the fall. These teleospores, upon germination in the spring, must infect the barberry before the rust can again attack the wheat plant. This relationship led to the campaign to eradicate the barberry. Large numbers of barberry bushes have been eradicated and this is believed to have aided in reducing the average annual loss from stem rust in the spring wheat belt. It is believed, also, that in some seasons the urediniospores, or summer spores of the red-rust stage, blow from the south in time to cause epidemics of stem rust. As these spores overwinter in the south, epidemics of stem rust might occur even after all barberry bushes have been eradicated. Such an epidemic occurred in Minnesota in 1927, when the estimated yield per acre of spring wheat was only 10.9 bushels. A very promising method for the control of stem rust of wheat is the production and utilization of resistant varieties. Even tho stem rust may be controlled effectually by other than breeding methods, other spring wheat diseases cause enormous losses.

Fusarial head blight, or scab, is another constant menace and may reduce average yields in the state as much as 10 per cent. It may be expected to increase in severity in the corn-growing sections of the state when wheat follows corn. The disease organism, *Gibberella saubinetii,* causing ear, stalk, and root rots of corn, is responsible also for scab of wheat. It may overwinter on cornstalks and stubble and attack wheat the following year. Some varieties of wheat are much more resistant to scab than others, Preston and Haynes Bluestem being highly resistant. Marquis is susceptible. The only known means of control is the use of resistant varieties.

Other diseases of importance are root rots, leaf rust, loose and covered smut, and black chaff. For each of these diseases there are both resistant and susceptible varieties. For example, Marquis is somewhat susceptible to root rot; certain hybrids that had Kanred win-

ter wheat as one parent appear resistant. Marquis is resistant to loose smut and to bunt, and somewhat resistant to leaf rust; Kota, a selection resistant to stem rust made at the North Dakota experiment station, is susceptible to all three diseases. Some hybrid wheats and most of the durums are much more resistant to leaf rust than Marquis; some new hybrids, such as Hope, are highly resistant to stem and leaf rust, bunt, and scab, but very susceptible to a disease known as black chaff, to which Marquis is resistant.

To add to the difficulty of obtaining resistance to a disease that may cause severe losses under environmental conditions favorable for the development of the organism, many organisms that cause disease are composed of physiologic races that have different infection capabilities. In stem rust of wheat, more than 60 races of rust have been identified that can be differentiated by their manner of infecting certain wheat varieties used as differential hosts. The breeding of resistant varieties, then, is resolved into the production of a variety resistant to all physiologic forms of the pathogene commonly found in a given area.

Three types of stem-rust resistance have been discovered—physiologic, morphologic, and functional.[2] Physiologic resistance is specific, certain varieties being resistant to one physiologic form of rust and susceptible to another. The rust gains entrance to the wheat plant but dies after relatively few cells have been killed. No bread wheat is available that has physiologic resistance to all races of stem rust. Morphologic resistance that is dependent upon some peculiar structure of the host plant is found in some varieties and might be expected to function against all physiologic races of the parasitic organism. Varieties with morphologic resistance often produce rust pustules, but these are much smaller than on varieties that lack this resistance and consequently do much less damage. Helen Hart recently discovered a new type of resistance which is dependent upon the time that the stomata open in the morning, for the germ tubes of the rust enter the wheat plant through the stomata. Varieties in which the stomata remain closed until several hours after sunrise are resistant. This type, known as functional resistance, might be expected to make a variety highly resistant to all physiologic forms of rust.

Besides resistance to disease, a new variety must have high yielding ability; desirable agronomic characters, such as strength of straw and freedom from shattering; and also satisfactory milling and baking qualities. No variety of spring wheat combining all these desirable qualities is available. If the northwest spring wheat area is to retain its

2 These studies have been made in Minnesota by E. C. Stakman and his co-workers.

leadership as a home of high quality wheat, and if spring wheat continues to be a paying crop for farmers, *new and improved varieties must be produced by the breeder.*

Spring Wheat Breeding Program

The breeding of spring wheat is a co-operative problem between the agricultural experiment stations in Montana, North Dakota, South Dakota, and Minnesota, and the Office of Cereal Crops and Diseases of the United States Department of Agriculture. While informal and formal co-operation between some of these agencies has been practiced for many years, the present program was undertaken as the result of a meeting held at Fargo, North Dakota, in March, 1928.

Besides co-operation between states and the United States Department of Agriculture, there is a co-operative attack by investigators within single states. In Minnesota, the Divisions of Agronomy and Plant Genetics and of Plant Pathology and Botany have united in breeding disease-resistant varieties. The project on genetics of rust resistance was formulated in 1916 and was merged with the co-operative project on disease resistance in 1921. Throughout this period the Office of Cereal Crops and Diseases has co-operated in this wheat-breeding program. The Division of Agricultural Biochemistry supervises the milling and baking trials of wheat in Minnesota, and conducts other tests of quality.

An outline of methods of breeding now in use may emphasize the nature of the problem. Wheat belongs to the self-pollinated group of crop plants. This means that each seed results, as a rule, from the union of an egg cell and a male reproductive cell from the same plant. Natural cross-pollination to the extent of about 4 per cent may occur in wheat at University Farm, and if such a natural cross occurs between plants of different varieties, eventually a new variety may result that has some of the differential characters of both parents. Occasional mutations, sudden changes in hereditary factors, may occur but they are so infrequent under ordinary conditions that a variety may be kept reasonably pure (homozygous) if cross-pollination is prevented.

All the progeny of a single self-fertilized homozygous plant have like hereditary characters. The differences observed in homozygous lines are a result of environmental influences and are not inherited. Because commercial varieties contain mixtures of types or recombinations from natural crosses, head or individual plant selection has been used as a means of isolating new varieties. The well known Kansas winter wheat variety, Kanred, was obtained by selection.

Selection as a method of varietal improvement.—The production of a new variety by selection may be summarized as follows:

First year, head or plant selection.—Several hundred selections, at least, should be made from a variety. The progeny of each head or plant is sown in a short row. All undesirable types (weak straw, etc.) are discarded on the basis of observation.

Second to fourth years, rod-row trials.—These consist of rows approximately 18 feet long (one foot being trimmed off each end at harvest). The rows are spaced one foot apart. Three rows, when seed is available, are grown for each plot, and the central row only is harvested for data on yield. Three such systematically distributed plots are used for each selection. In Minnesota the rod rows are grown at the Central station, at University Farm, St. Paul, and at the Branch stations at Waseca, Crookston, and Morris. Yield determinations are made and notes are taken on agronomic characters and disease reaction. Milling and baking studies are made, using a mixture of seed from the several localities. After a three-year trial, the 6 or 8 best selections are increased and sown in $\frac{1}{40}$ acre plot trials.

Fifth to seventh years, $\frac{1}{40}$ acre plot trials at University Farm and Branch stations.—Three systematically distributed plots are used for each variety in a trial. Notes are taken similar to those taken for the rod-row tests, and individual milling and baking studies are made. The yield data are analyzed on the basis of biometrical methods.

Eighth year, increase of any promising sort and distribution through the Minnesota Crop Improvement Association.—Each winter all experiment station men interested in crops meet and, on the basis of data collected, decide what new varieties, if any, shall be distributed to farmers.

Crossing as a method of wheat improvement.—The selection method isolates new types from mixed populations. The purpose of crossing is to combine in a single variety the desirable characters of two or more varieties and eliminate the undesirable characters. It is the direct application of the Mendelian Law of Heredity to crop improvement. Most important crop characters are dependent for development upon several hereditary factors, or genes. Environment does not change these factors—it does condition their development.

An illustration indicates how new varieties can be produced by crossing unlike varieties. Each differential character is dependent upon only a single factor pair and complete dominance of one character over the other will be hypothecated. As the parents used for the cross were homozygous, the zygotes, i. e., the individual parent plant or plants, each contain a duplex condition for each gene or factor. When

the reproductive cells are produced, the genetic condition for each character is reduced to the simplex condition. The genetic factors are located in a linear manner in the chromosomes and in a certain definite location, and each crop plant and wild species has a definite number of chromosomes. In bread wheat there are 21 pairs of chromosomes and in durum wheat 14 pairs. When the gametes, or reproductive cells, are produced, there is a reduction in chromosome number to half that carried in the body cells.

The laws of heredity cannot be exemplified in detail here. An illustration will be given, altho in many crosses the conditions will be more complex, i. e., more factors will be necessary to explain the results for each differential character or more characters will be studied than in the illustration. The parents are Marquis, which is an awnless (tip awned) wheat susceptible to stem rust; and Hope, a bearded variety resistant to stem rust. In the cross there is a dominance of the awnless condition over the bearded and of resistance to rust over susceptibility. The type desired is awnless and resistant to stem rust. Allowing the dominant condition of a factor to be represented by a capital letter and the recessive condition by a small letter, the factorial relation of these varieties may be represented as follows:

Marquis		Hope	
Awnless	AA	Bearded	aa
Susceptible	rr	Resistant	RR
First generation plants, F_1		AaRr (awnless and resistant)	
Gametes of F_1		AR, Ar, aR, ar, in a 1:1:1:1 ratio	

The recombination of F_1 gametes to produce zygotes is illustrated by the use of the Punnett square:

		Male Gametes			
		AR	Ar	aR	ar
Female gametes	AR	AARR	AARr	AaRR	AaRr
	Ar	AARr	AArr	AaRr	Aarr
	aR	AaRR	AaRr	aaRR	aaRr
	ar	AaRr	Aarr	aaRr	aarr

It will be noted that the homozygous types which will breed true, i. e., produce progeny with hereditary characters like their immediate parents, AARR, AArr, aaRR, and aarr, are located on the diagonal in the above illustration. The numbers in each of the nine different classes and their breeding habit are given in the following description:

F_2 plants*	Zygotic formula	Breeding habit in F_3
9 awnless resistant	1 AARR†	Will breed true for awnlessness and resistance.
	2 AaRR	Will segregate for beards vs. awns and breed true for resistance.
	2 AARr	Will breed true for awnlessness and segregate for disease reaction.
	4 AaRr	Will segregate for both characters.
3 awnless susceptible	1 AArr	Will breed true for awnlessness and susceptibility.
	2 Aarr	Will breed true for susceptibility and segregate for awns vs. beards.
3 bearded resistant	1 aaRR	Will breed true for beards and resistance.
	2 aaRr	Will breed true for beards and segregate for disease reaction.
1 bearded susceptible	1 aarr	Will breed true for beards and susceptibility.

*F_1, F_2, etc., stand for 1st, 2nd, etc., filial generation.
† The type desired.

It will be observed that the first generation of the cross was awnless, altho the tip awns are slightly longer than those of Marquis; and that the plants are resistant to stem rust as is the Hope parent. The reproductive cells, or gametes, of the F_1 are of four sorts, two like the parents and two with a new combination. The hereditary characters of the male and female reproductive cells are of like nature. The four types of gametes recombine to produce nine different hereditary combinations of factors in F_2. The type marked with a †, AARR, will breed true for awnless habit and resistance to stem rust, as it is duplex for the determiners of genes for both these characters. Thus, on an average, in F_3 one line out of every 16 will breed true for the characters desired.

The production of a new variety by hybridization and subsequent selection may be summarized as follows:

First year.—Make the cross between parents selected because of their known characters. If a greenhouse is available, the F_1 generation may be grown the same year the cross is made.

Second year.—Grow several thousand plants spaced so that individual plants can be studied. If disease reaction is of interest, it is

desirable to obtain an artificial epidemic of the disease or diseases to be studied. For spring wheat, stem and leaf rust infection is studied in one nursery and reaction to such diseases as scab, bunt, and root rot in another. Notes on black chaff are taken in both nurseries. Resistant plants that appear desirable in other characters are threshed individually and selection is made on the basis of seed characters.

Third to fifth year.—Progeny lines of 25 to 50 plants are grown in the disease gardens, each line consisting each year of the progeny of a single plant. The seeds are spaced in the row so that individual plants can be studied. By the fifth generation some of the lines will appear homozygous (all plants alike) for disease reaction and of like habit for other characters, such as the presence of beards, height of plant, and time of maturity. These lines are harvested by the individual plant method and threshed individually, and the grain is examined. If all plants produce grain of like shape, color, and type, the seeds of all plants of the line are bulked and furnish the material for a yield trial.

Sixth to eighth year.—Yield trials are conducted in rod-rows. Further tests of agronomic characters and reaction to disease and preliminary milling and baking trials are carried out as with selections.

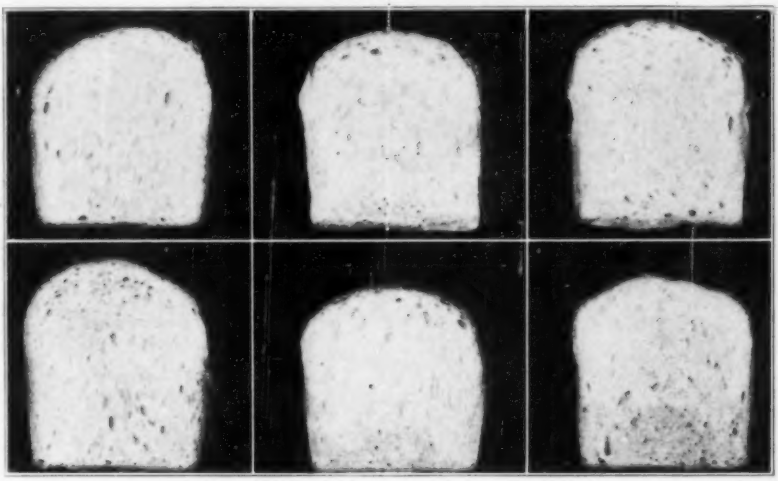
Ninth to eleventh year.—$\frac{1}{40}$ acre plot trials and milling and baking tests are carried out as with selections.

Twelfth year.—Increase of the best sort for distribution.

Methods of studying milling and baking quality.—To obtain a new variety with desirable agronomic characters and resistance to the diseases that attack the spring wheat crop is a difficult task. The wheat breeder faces the equally difficult task of obtaining a new variety with satisfactory milling and baking qualities. Resistance to disease can be studied in very small plots, where only a few plants are grown. Such characters as plumpness, color, and texture of grain can be studied and selection for satisfactory quality made when only a few plants are available in each progeny line. Milling and baking quality can be estimated from actual studies where small quantities of grain are milled and baked in comparable trials. For these studies it is necessary to use the chemical and testing laboratories and the work of the breeder is supplemented by that of the cereal technologist.

An accurate estimate of milling and baking quality is impossible until several pounds of seed are available. A new variety of wheat may be satisfactory in all characters except milling and baking quality and it is impossible to learn this until after several seasons of study. In producing new varieties by crossing unlike sorts, sufficient seed for milling and baking tests is not available until the sixth year after

the cross is made. This greatly complicates the work of the breeder. A large amount of material inferior from a milling and baking standpoint must be carried for several years before it can be tested and discarded. The only method now available for overcoming this difficulty is to carry many lines and then discard those that by actual trial are inferior in milling and baking qualities. Breeding work would be greatly simplified if methods could be devised of making accurate estimates of quality from only a few grams of seed.



Loaves Made in Baking Trials in 1929 from a Mixture of Equal Quantities of Grain from University Farm, Waseca, Crookston, and Morris.

Upper row, left to right: Marquis, Marquillo, Ceres.

Lower row, left to right: A Marquis-Kanred hybrid used as one parent in crosses with Marquillo to produce new lines, which are now in rod-row trials; Hope; Marquis x Emmer, also called H-44.

Rod-row trials are made in Minnesota at University Farm, Waseca, Crookston, and Morris. A mixture of an equal quantity of grain from each locality is used for the preliminary milling and baking trial. By this method sufficient seed is available, about 6 pounds being necessary for each trial. Data are taken on protein content; percentage of total flour; water absorption; color, texture, and volume of loaf. Extremely undesirable types are eliminated on the basis of this study.

The varieties grown in the $\frac{1}{40}$ acre plot trials have proved promising in the rod-row trials or are desirable varieties produced elsewhere. Comparative trials in $\frac{1}{40}$ acre plot tests are made at University Farm, Waseca, Morris, Crookston, Grand Rapids, and Duluth. Sufficient seed is available from each variety so that milling and baking qualities can be studied with the grain obtained from each field trial.

Before a new variety is distributed, its milling and baking qualities are determined from these studies.

Present Status of the Problem

The conference at Fargo, North Dakota, on March 27, 1928, placed the spring wheat breeding program on a definite co-operative basis. Co-operative breeding projects were at that time being conducted in Minnesota, Montana, and North Dakota between state agencies and the Bureau of Plant Industry, United States Department of Agriculture. Space is not available to discuss the work in detail.

The earliest spring wheat breeding investigations were initiated by W. M. Hays, before 1900. They were conducted at the North Dakota and the Minnesota experiment stations. Two new varieties were produced by selection and were introduced into culture. These are Haynes Bluestem, Minn. 169; and Fife, Minn. 163. They were grown widely, but were superseded by Marquis about 1912. Marquis wheat, which is of hybrid origin, was produced in Canada. It matured earlier than Bluestem and often escaped stem rust when Bluestem was severely injured. After Marquis had become the chief variety in the spring wheat area, it appeared to suffer as severely from stem rust as Bluestem had previously. This led to renewed activity to obtain a rust-resistant variety by breeding methods, and an attempt to reduce the danger of stem rust epidemics by an intensive campaign of barberry eradication. Other wheat diseases have increased in severity and now the obtaining of disease-resistant sorts is the first essential of the breeding program. Important diseases include stem and leaf rust, covered smut (bunt), loose smut, scab, root rots, and black chaff, as mentioned before.

During the early years of study, when stem rust was believed to be the chief limiting disease, the only rust-resistant varieties available belonged to the durum group. A close association of characters in crosses of durum and common wheats greatly increases the difficulty of obtaining the desired recombination of characters. Marquillo, the new variety that is being increased by approved growers in Minnesota, was produced from a durum-common cross. It obtained its rust resistance from the durum parent, Iumillo; and its milling and baking qualities from Marquis, the common-wheat parent. Its chief faults are susceptibility to root rots, altho in this regard it is not markedly inferior to Marquis; and its slight tendency to produce a yellowish loaf. It has been used as a parent in further crosses and some of the hybrids obtained from the double cross of Marquillo x Kanred-Marquis selections appear much superior to Marquillo, altho further tests are neces-

sary before any of these new hybrids are ready for distribution. The more promising hybrids from this cross are being grown in Minnesota for the first time in 1929, in $\frac{1}{40}$ acre plots, altho they have been tested extensively in rod-row trials for three years. Some of the most promising are being grown in rod-row trials in North Dakota and Montana.

Kota is a variety of bread wheat selected in North Dakota. It is resistant to stem rust but is susceptible to leaf rust, bunt, and loose smut. An improved variety, Ceres, has been produced in North Dakota from a cross of Kota and Marquis. Its milling and baking qualities are satisfactory, it is resistant to stem rust, but is somewhat susceptible to scab and to the smuts. Other selections from Marquis-Kota crosses that appear superior to Ceres are under trial in North Dakota. Some of these are believed to be considerably superior to Ceres in many respects.

No statement of the present status of the problem would be complete without speaking of the work of E. S. McFadden, in South Dakota, who has succeeded in transferring the resistance to stem and leaf rust, bunt, and smut to a common wheat variety from a cross of Emmer with Marquis. Two new selections should be mentioned. These are Hope and a sister selection, H-44. In most trials these two selections have proved very resistant under field conditions to stem and leaf rust, bunt, and loose smut, and to show fairly satisfactory milling and baking qualities. They both appear very susceptible to black chaff. In nearly all spring-wheat crosses made since their production, either Hope or H-44 has been used as one parent. No new varieties are available that have been produced from these crosses. Some selections, however, have been studied under disease epidemic conditions and appear very promising.

Conclusion

Plant diseases are one of the chief causes of the great seasonal variability in the yield of spring wheat. New and improved varieties must be produced if spring wheat continues to be a profitable crop. The most satisfactory general method of controlling diseases is the production and introduction of resistant varieties. The problem is difficult, because resistance to several diseases is essential and because of the difficulty of combining a considerable number of characters, including desirable milling and baking qualities, in a single variety. The progress already made is an indication, however, that the problem can be solved satisfactorily by this method.

CONCERNING THE NATURE OF THE PROTEIN EXTRACTED FROM WHEAT FLOUR BY HOT ALCOHOL[1]

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Introduction

The characterization and classification of the wheat proteins as established by the investigations of Osborne (1907) have served as the basis for nearly all subsequent researches concerning these proteins. Differences among the bread-making characteristics of various wheats and flours are generally ascribed to variations in both quantity and quality of gluten. Consequently, cereal chemists have persistently sought to improve the accuracy and reliability of existing methods for the quantitative separation and estimation of individual gluten proteins by modifying the established procedures and, in certain instances, devising new ones. A lack of precise and reliable analytical methods may be largely responsible for the fact that the fundamental cause and nature of variations in "gluten quality" have never been clearly established.

Altho for purposes of general consideration and discussion the flour proteins may be characterized and classified on the basis of their peculiarities of solubility in various reagents, it has been repeatedly shown that the mere extraction of flour with various solvents will not ordinarily accomplish a sharp and clearly defined *quantitative* separation of these proteins. Factors contributing to the unreliability of alleged quantitative separations of flour proteins by means of solvents have been investigated and reported by Kjeldahl (1896), Teller (1896), Chamberlain (1906), Greaves (1911), Olson (1913), Bailey and Blish (1915), Sharp and Gortner (1923), Sharp and Herrington (1927), Gortner (1927), and others.

Bailey and Blish (1915) undertook to investigate specifically the magnitude of errors due to "overlapping" solubilities, which are always involved in the use of certain of the conventionally adopted protein solvents. Their work included an attempt to ascertain both the amount and the nature or identity of protein material extracted from flour by alcohol of varying strengths. They were especially concerned with the *identity* of the protein material extracted, as it had been repeatedly

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established that different concentrations of alcohol will extract different *amounts* of gliadin from almost any given flour. They found no conditions under which gliadin only was extracted by alcohol, but all alcoholic extracts apparently contained appreciable quantities of either albumin or globulin or both. The amount of "albumin-globulin" fraction was estimated by measuring the ammonia nitrogen produced by acid hydrolysis of the entire extract, in terms of percentage of the total nitrogen. As pure gliadin, on hydrolysis, yields 25.5% of its nitrogen as ammonia, and as albumin and globulin yield 6.8% and 7.7% respectively, the percentage of ammonia nitrogen produced by the hydrolysis of a mixture of these proteins was regarded as indicative of the percentage of gliadin in the mixture. They found a maximum quantity of protein extracted by 50% alcohol in pressure flasks at 83-84°C., which is approximately the temperature at which 50% ethyl alcohol boils under atmospheric conditions. The percentage of ammonia nitrogen in the hydrolyzed protein extracted by hot alcohol indicated practically as high a percentage (92.4) of gliadin in the extract as was found when extractions were made with 50% and 70% alcohol at room temperature. As the hot alcohol removed the maximum quantity of gliadin without any apparent increase of other proteins in the extract, Bailey and Blish (1915) stated "extraction with 50 per cent alcohol at 83° to 84° for 3 hours apparently effected a complete separation of the gliadin, and this method is recommended for this purpose."

Nasmith (1904), Hoagland (1911), Greaves (1911), and more recently Sharp and Herrington (1927) also reported that hot alcohol extracts more protein from flour than does cold alcohol. The experiments of Sharp and Herrington (1927) show clearly that boiling 50-60% alcohol extracts more protein than 70% alcohol, and that for any given alcoholic concentration more protein is removed by boiling alcohol than by alcohol at room temperature. They state that the increased amount of protein removed by hot alcohol "indicated a peptizing action on non-gliadin protein material" but that "if the additional amount of protein removed by extracting with hot alcohol over that obtained by extracting with cold alcohol is gliadin, then the method of Sharp and Gortner (1923) for glutenin . . . should be modified to extract with hot alcohol."

It is of interest as well as of importance to know definitely whether or not the additional amount of protein extracted by hot alcohol is gliadin or non-gliadin protein. If it is gliadin, then the methods that have ordinarily been employed for the quantitative estimation of gliadin

and glutenin, respectively, are inaccurate and unreliable. The method of Sharp and Gortner (1923) for glutenin must be regarded as reasonably accurate and reliable if one adheres to Osborne's characterization and classification of the wheat proteins. That method provides for the extraction of a sample of flour, first with salt solution and then with 70% alcohol at room temperature. The total amount of protein extracted by both procedures, subtracted from the total protein in the flour, represents glutenin. Two other methods for the quantitative estimation of glutenin, one proposed by Blish and Sandstedt (1925) and the other by Blish, Abbott, and Platenius (1926), give results that are usually in agreement with those obtained by the method of Sharp and Gortner (1923). If boiling alcohol were used in the procedure of Sharp and Gortner (1923) much larger percentages of gliadin, and correspondingly smaller percentages of glutenin than are usually found, would be indicated. If this is true, then the present method of Sharp and Gortner (1923) is inaccurate, as are also the two methods proposed, respectively, by Blish and Sandstedt (1925) and by Blish, Abbott, and Platenius (1926). On the other hand, if the above-named methods are accurate, or reasonably so, Bailey and Blish (1915) must have been mistaken in their apparently well-founded belief that the protein material extracted by hot alcohol, in addition to exceeding in quantity that which is removed by cold alcohol, contains no greater percentage of non-gliadin protein than does the cold alcohol extract.

As previously noted, Bailey and Blish (1915) based their estimates as to the identity of the extracted protein material upon the ammonia nitrogen produced after complete acid hydrolysis of the entire extract, as pure gliadin yields 25.5% of its total nitrogen as ammonia nitrogen, whereas albumin and globulin yield 6.8 and 7.7%, respectively. However, Blish and Sandstedt (1925) based their criteria of the validity of their glutenin method upon similar grounds. Here is a decided incompatibility, with the evidence on the one hand favoring the use of hot alcohol for complete extraction of gliadin; that on the other hand, of the *same nature* arguing *against* the use of hot alcohol.

In an effort to explain this anomalous situation, the writers have made further inquiry into the nature of the protein that hot alcohol extracts from flour in excess of the amount removed by cold alcohol. The work was designed thoroly to test the possibility of a peptization of non-gliadin material by the hot alcohol. The findings of Bailey and Blish (1915) argue strongly against this possibility, for the admixture of non-gliadin protein would be expected to reduce the magnitude of the

figure for ammonia nitrogen accordingly, as the non-gliadin proteins of flour all yield markedly lower percentages of ammonia set free by acid hydrolysis. If hot alcohol peptizes some of the non-gliadin protein, why did this not lower the figure for ammonia nitrogen that Bailey and Blish (1915) obtained upon hydrolysis of their hot alcohol extract?

Blish (1927) made a preliminary statement as to the nature of the "extra" protein extracted from flour by boiling 70% alcohol. He states, "without giving details, which will be published later, it may be said that this protein material is, in all probability, a product resulting from the splitting off of a certain portion of the glutenin molecule by the hot alcohol treatment." This report undertakes to present and discuss the evidence upon which the foregoing statement is based.

Experimental

Preliminary experiments were designed to indicate whether the gluten or non-gluten portion of the total flour protein is the source of the excess protein extracted by hot alcohol over the amount removed by cold alcohol. Crude gluten was prepared from a sample of baker's flour, by washing out in the conventional manner. This was dried in vacuo at 66°C., and ground to a powder, which contained 82% protein. When extracted by vigorously shaking 2 gm. for 3 hours with 200 cc. of 70% alcohol at room temperature, the clear filtered extract was found to contain 52.8% of the total protein originally in the gluten. However, when 2 grams of the dry gluten was extracted for one hour by 200 cc. *boiling* 70% alcohol under a reflux condenser, 67.8% of the total protein was found in the extract.

As the greater portion of the non-gluten proteins (albumin and globulin) was removed during the preparation of the gluten, they are virtually eliminated from consideration in the foregoing experiment. It then appears that the excess protein extracted by hot alcohol probably originates largely from that portion of the flour protein usually regarded as glutenin.

In the next series of experiments, two 120-gram portions of a high-protein hard winter wheat flour were each extracted with 2 liters of 5% potassium sulfate, to take out the "soluble proteins," and later with distilled water to remove the salt. Portion A was then subjected to prolonged extraction, with thoro and frequent shaking, with 70% alcohol at room temperature. Portion B was extracted with 2 liters of *boiling* 70% alcohol for 3 hours on a steam bath, under a reflux condenser. Fifty cc. of the extract from portion A contained 0.0238 gm.

nitrogen, or 0.1357 gm. protein (N x 5.7). Fifty cc. of extract from portion B contained 0.031 gm. nitrogen, or 0.1767 gm. protein (N x 5.7).

When the two extracts were separately hydrolyzed with strong hydrochloric acid, portion A yielded 25.7% of its total nitrogen as ammonia nitrogen and portion B, 26.1%. The question again arises: Is the extra protein dispersed by the hot alcohol due to the peptization of glutenin, or to a portion of gliadin that is not extractable by cold alcohol? If caused by peptization of glutenin, why is not the ammonia nitrogen value accordingly lowered in the hot alcohol extract, since Osborne and Harris report the percentage of ammonia nitrogen in glutenin as 18.9, while Cross and Swain (1924) report ammonia nitrogen percentages in a number of glutenins, ranging from 12.88 to 16.15, and Blish (1916) records 16.5 and 16.17, respectively, in two preparations?

In an attempt to secure further information on these points, the residue from the extraction of portion B with hot alcohol was washed by decantation with 65% alcohol, until the washings were protein-free, and a second extraction with boiling 70% alcohol was conducted upon the residue, using 2 liters of alcohol again, and boiling for 3 hours under the reflux condenser. As much of this extract as was convenient was decanted, the alcohol was removed by evaporation, and the residue was hydrolyzed with acid. The total nitrogen in this hydrolysate was 0.118 gm., representing 0.673 gm. of protein (N x 5.7). This is less than the entire amount of protein actually dispersed by the second extraction with hot alcohol, being only the amount in the decanted portion of the extract. The percentage of ammonia nitrogen in this extract, after hydrolysis, was 23.5, which still approximates more nearly the figure for gliadin than that for purified glutenin.

A third extraction of the residue with boiling 70% alcohol removed an additional amount of nitrogen, equivalent to about 0.4 gm. protein. On hydrolysis, this material yielded 20.24% of its total nitrogen as ammonia nitrogen, a figure still in considerable excess of recorded values for pure glutenin.

In order to ascertain approximately the amount and nature of the protein material remaining after the three extractions with boiling 70% alcohol, the residue was washed with cold dilute alcohol, and then extracted with sodium hydroxide in alcohol, according to the procedure of Blish and Sandstedt (1925). When the major portion of this extract was decanted and hydrolyzed, the hydrolysate was found to

contain only 0.0574 gm. nitrogen (equivalent to 0.327 gm. protein), of which 20.1% was estimated to be ammonia nitrogen, a percentage that is still higher than the corresponding figure for pure glutenin.

The foregoing experiments appear to establish several facts, among which are the following:

1. When flour is repeatedly extracted by successive portions of boiling 70% alcohol, there is no stage at which extraction of nitrogenous matter is completed, and each successive treatment "extracts" additional protein material. None of the protein fractions produced by this treatment show as low percentages of ammonia nitrogen after acid hydrolysis as the highest values recorded for glutenin. This permits of the strong suspicion that "glutenin" itself is perhaps to be regarded as a derived rather than a naturally occurring protein, and that it may be merely a "fraction" produced by the action of dilute alkali upon flour or gluten.

2. There is no evidence tending to show that any reliable quantitative separation of individual wheat proteins may be effected through the use of hot alcohol.

3. Boiling alcohol has a "peptizing" action upon that portion of the flour protein generally classified as the *glutenin* fraction and that has been customarily regarded as a distinct chemical individual.

4. This so-called "peptization" of the glutenin apparently involves some alteration, disintegration, or rearrangement whereby the peptized portion yields a greater percentage of ammonia upon hydrolysis than is found in supposedly "pure" preparations of glutenin.

5. This has led, as in the work of Bailey and Blish (1915), to the drawing of erroneous conclusions regarding the identity of the "extra" protein extracted by hot alcohol; these conclusions have been based upon the percentages of ammonia nitrogen yielded upon hydrolysis of the extracts.

6. Extraction with hot alcohol *cannot* properly be recommended as a method for the complete separation of gliadin from glutenin, and is therefore undesirable as a procedure for the quantitative estimation of gliadin.

Extraction of Glutenin with Hot Alcohol

In order to study more specifically the effect of boiling 70% alcohol on glutenin alone, a sample of purified glutenin was subjected to treatment, and nitrogen distributions by the Van Slyke procedure were determined respectively in the glutenin before extraction, in the extract itself, and in the residue after extraction. A quantity of glu-

tenin was prepared and purified according to the principle of the method proposed by Blish and Sandstedt (1925). Twenty gm. of this glutenin was extracted with one liter of boiling 70% alcohol for 3 hours under a reflux condenser on a water bath. The extract was removed as completely as possible by decantation, a fresh liter of 70% alcohol was added to the residue, and a second extraction at boiling temperature was performed. The process was repeated until the glutenin had been subjected to six extractions with boiling 70% alcohol. Aliquot portions of each extract were taken for total nitrogen determinations. The amount of protein (N x 5.7) removed by these extractions, respectively, is shown in Table I.

TABLE I

AMOUNTS OF PURE GLUTENIN PEPTIZED BY SUCCESSIVE EXTRACTIONS WITH BOILING 70% ALCOHOL

Extract No.	Protein (N x 5.7) in extract*
	per cent
1	9.8
2	6.2
3	4.3
4	2.1
5	1.9
6	1.6
Total	25.9

* Expressed as percentage of the original 20 gm. of glutenin.

It appears from Table I that nearly 10% of the glutenin was "peptized" by the first extraction with hot alcohol, with decreasingly smaller percentages dispersed by subsequent treatments, a total of 25.9% having been dispersed by the six treatments. Nitrogen partition (Van Slyke) was determined in (1) the original glutenin, (2) the extract from the first treatment, (3) the combined extracts 2 to 5, and (4) the residue after six extractions, with results as shown in Table II.

TABLE II

NITROGEN DISTRIBUTION IN ORIGINAL GLUTENIN AND IN FRACTIONS PRODUCED BY TREATMENT WITH BOILING 70% ALCOHOL, SHOWN AS PERCENTAGE OF TOTAL NITROGEN

	Original glutenin	Extract No. 1	Combined extracts 2-5	Residue
Ammonia N	12.40	16.90	20.95	9.54
Humin N	1.05	0.80	0.12*	0.92
Arginine N	11.18	10.55	7.34	11.95
Cystine N	0.40	0.20	0.12	0.30
Histidine N	7.00	1.73	4.22	7.08
Lysine N	6.80	4.80	3.94	8.17
Mono-amino N in filtrate	55.10	53.50	50.20	56.10
Non-amino N in filtrate	5.85	11.50	11.50	5.60
Total	99.78	99.98	98.39	99.66

* Most of the humin was lost.

The data in Table II justify the conclusion that the material "peptized" by the treatment of glutenin with hot alcohol is of chemical constitution that differs appreciably from that of either "pure" glutenin or gliadin, as these proteins have been characterized according to the work of Osborne (1907). It is of interest to note that altho the peptized material differs from the original glutenin in this respect, the *residue* after successive treatments with hot alcohol shows nearly the same composition, estimated by the Van Slyke procedure, as the original glutenin; the single exception being a slight reduction in the ammonia nitrogen fraction. Aside from these items, the data do not permit the drawing of definite conclusions. They do not afford any reliable explanation, to the writers at any rate, of *how* and *why* the observed effects have been produced.

The experiments offer a convincing confirmation of the stated belief of Sharp and Herrington (1927) that treatment with hot alcohol effects "a peptizing action on the non-gliadin protein material," and they establish the fact that glutenin is the non-gliadin protein material involved.

Many workers have used hot alcohol in the preparation and purification of alleged "individual" cereal proteins. Osborne (1895) extracted barley flour with hot 75% alcohol, in an apparent belief that this treatment did not alter the glutelin. Johns and Brewster (1916) prepared a prolamine from kafir seeds by extraction with hot alcohol; Hoffman and Gortner (1925) prepared prolamines from both kafir and sorghum by a similar process, and Hoffman (1925) isolated an "alcohol soluble" protein from rice by the same procedure. Hoffman (1925) does not believe his alcohol-soluble protein is a part of the rice glutelin, as no more could be obtained upon additional treatment with hot alcohol. This, however, cannot be accepted as conclusive evidence that the prolamine obtained by boiling alcohol is a naturally occurring and not a derived protein substance. Perhaps the latter observation may be applied with equal force to all cereal protein preparations.

In any event the experiments herewith reported clearly justify a belief that cereal protein preparations involving the use of hot alcohol are open to the suspicion that they may have been to a greater or less degree contaminated with "fractions" of proteins other than the one whose exclusive isolation was intended. There is need for further critical study of this situation. It appears more than likely that "glutenin," as ordinarily prepared by the use of dilute alkali, is not a naturally occurring individual protein, but merely a "fraction" derived

through the action of the alkali, and varying somewhat in composition according to the strength of the alkali used, as well as with the time and temperature of extraction. Additional evidence on this point will be presented in a later communication.

Conclusions

1. Boiling 70% alcohol not only disperses or dissolves the gliadin of wheat flour, but it exerts a very appreciable "peptizing" action upon the glutenin.

2. Hot alcohol is not a suitable reagent for use in the quantitative estimation or isolation of wheat gliadin.

3. The use of hot alcohol for isolating or purifying any of the cereal proteins is of doubtful validity, and is open to serious question.

4. It appears likely that "glutenin" is a derived rather than a naturally occurring protein substance.

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RELATION OF HYDROGEN-ION CONCENTRATION AND BUFFER VALUE TO THE BAKING QUALITY OF FLOUR. PART II

A Correction

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(Received for Publication Sept. 16, 1929)

In our paper of the above title (Fisher and Halton, 1929) we pointed out that the gassing power of a flour is independent of its "strength" and that "so far as the gassing power of a flour is under the control of the miller and baker, it should be regarded as a separate problem from strength." "This isolation of gassing quality for separate study and its elimination altogether from the problem of strength is perhaps the biggest positive advance that has yet been made in the study of strength. This advance was due to A. E. Humphries, who has emphasized the point of view for many years in the numerous Reports of the Home Grown Wheat Committee of the National Association of British and Irish Millers; it was clearly stated by Humphries in 1911 and again in 1920."

It has been pointed out to us that the above statement is not historically correct. The isolation of gassing quality as a separate factor in "strength" arose directly out of the work of T. B. Wood published in 1907 (Wood, 1907). The original paper must be consulted for details of the work; the following quotations establish sufficiently the historical point involved:

"The capacity of a flour for giving off gas when incubated with yeast and water is the factor which in the first instance determines the size of the loaf."

"So far as I am aware this factor has not been suggested as one of the components of strength by any investigator." (p.153)

"The gas which influences the size of the loaf is that which is given off during the later stages of fermentation, when the loaves have been moulded and are waiting to go into the oven." (p.156)

"This gas produced in the later stages of fermentation comes rather from sugar formed by diastatic action than from added sugar, or sugar originally present." (p.157)

Wood carried out his investigations in close association with Humphries and the importance of Wood's original conception and work was repeatedly emphasized by Humphries in the Reports of the Home Grown Wheat Committee (already referred to) from 1907 on. This line of investigation was subsequently extended and developed with far-reaching results by Humphries (See Humphries and Simpson, 1909, and Humphries, 1911 and 1920). In particular, Wood's original idea had to be modified materially in the light of later experience. The quantity of gas given off in final proof does not determine the size of the loaf; it is rather a necessary condition for the production of a large loaf. It is obvious that a large loaf cannot result if the gas production during proof is inadequate. Sufficient gas must be produced; then, *if the other contributing factors are present,* a loaf of large volume may be made. Gas production, through the work of Humphries, has been brought largely under the control of the miller and the baker. This isolation of gas production for separate study *and control* constituted an advance of the greatest importance in the study of the strength problem.

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FERMENTATION TOLERANCE[1]

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(Read at the Convention, May, 1929)

The cereal chemist has repeatedly discovered new tests designed accurately to determine flour quality, only to learn that he had merely some new figures whose interpretation was not yet entirely clear.

The determination of fermentation tolerance, however, is not a new discovery, but a slow outgrowth of simple baking tests in which only one set of conditions is used for each flour. It undoubtedly gives, from some standpoints, information more valuable than that of any other test of flour quality yet devised, but it is by no means free from danger of misinterpretation.

Let us consider, for instance, a flour such as is illustrated by A, Figure 1. The loaf volume increases rapidly up to about 3 hours fermentation, reaches a pronounced peak at 3½ hours, and falls off sharply after 4 hours. This gives a picture of what this flour will do *under the conditions of this experiment.* But suppose the bakery that is to use it uses much less yeast and a longer fermentation period, or perhaps uses a sponge-and-dough method. Immediately the accuracy of the determination is lost. We may predict that this flour will be satisfactory because another giving similar laboratory results was satisfactory, but we cannot be absolutely certain of the results or of the best treatment for the flour.

If we study carefully the reasons for the form of the curve, especially the reasons for its falling off, and learn to analyze the factors responsible for it, we may be able ultimately to foretell accurately not only the behavior of the flour, but also the treatment required to give the best results of which it is capable.

Gas Production and Gas Retention

The first step in a study of this kind is a consideration of the two chief factors in the production of good volume and texture in bread—the rate of gas production while the dough is rising in the pan and, more important, the physical condition of the dough during this rise and the oven spring immediately following. The necessity for separating these two factors was stated as early as 1911 by

[1] Contribution No. 37, Dept. of Milling Industry, Kansas Agr. Expt. Station.

Humphries, in England, but in this country its importance has not been recognized. In fact, no attention has been paid to gas production except as it is related to diastatic activity.

The importance of gas production in evaluating different flours is illustrated in Figure 1. The solid line A represents the fermentation tolerance of a flour, as determined by mixing the dough with 3 per cent yeast, 1.8 per cent salt, 4 per cent sugar, and 1½ per cent shortening. Loaves were scaled off to correspond to 100 grams of flour, and given the fermentation indicated. Gas production is indicated by the broken line B, and was determined upon an equal portion of the same dough.

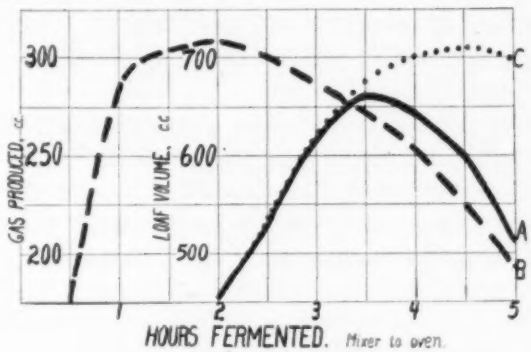

Fig. 1. Hard Wheat Flour
A. Loaf volume as affected by fermentation time
B. Gas produced, cc. per half-hour period
C. Loaf volume possible with adequate gas production

It is evident that the rapid falling off in gas production after 4 hours may easily account for the decreasing loaf volumes obtained after that time. This view is supported by the fact that the feel of the dough was still good even after 5 hours. Also, as shown by other determinations, this flour will produce loaf volumes above 700 cc., with even silkier texture, when baked with 2 per cent yeast and a long fermentation, or when baked by a sponge-and-dough method. The line C, of Figure 1, is based on these facts. It shows what the flour might be expected to do if gas production were kept up. The curve of fermentation tolerance apparently does not tell the full possibilities of the flour. Better volume and texture probably may be obtained either by changing the fermentation method so that the peak of gas production will coincide with the peak of dough quality, or by some change that will hasten conditioning without materially changing the gas curve, thus bringing the peak of dough quality forward to coincide with the peak of gas production.

Figure 2 shows the fermentation tolerance, A, and the gas production, B, of a soft wheat flour. As loaf volume begins to fall rapidly while gas production is still adequate, we assume that the curve of fermentation tolerance expresses practically the full possibilities of the flour. But even in this case we must consider that some addition, such as milk, malt, calcium salts, or oxidizing agents, might improve it.

Gas Production

We have some understanding of the factors governing gas production. We recognize that for its metabolism yeast requires sugar; simple nitrogenous substances such as amino acids or ammonium salts; certain mineral elements, particularly phosphates; and two substances of relatively recent discovery, Bios I and Bios II. The last two have too often being ignored, as white flours, especially patent flours, are likely to be deficient in them.

But even tho we supply the yeast with sufficient food and growth stimulants of whatever kind, gas production falls off after a time because of the accumulation of some of the end-products of fermentation, the most important of which are probably ethyl alcohol, the higher alcohols, and other substances grouped under the term "fusel-oil."

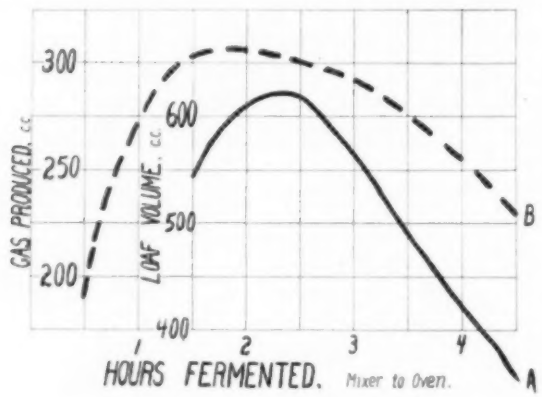

Fig. 2. Soft Wheat Flour
A. Loaf volume as affected by fermentation time
B. Gas produced, cc. per half-hour period

If a dough, the gas production of which is being measured, is worked down at intervals, a great increase in gas production will be observed in the 10 or 15 minutes immediately following, which often has not entirely disappeared after 25 or 30 minutes. The following appears to be a plausible explanation of this: During fermentation a scarcity of food and an excess of by-products have developed in the immediate vicinity of the individual yeast cell, and the diffusion of food toward the

cell and of end-products away is hindered because gas production has left only thin films of dough touching the yeast cell. The kneading down of the dough brings fresh food in contact with the cell and distributes the excess of end-products.

Gas Retention

Nothing is more obvious to the baker than that rapid gas production alone will not insure good bread. If the dough is too "young" or too "old" and gas production is adequate, the dough will usually rise normally in the pan, but will fail to rise properly, will perhaps even fall, when put into the oven. The physical condition of the dough must be such that the tiny gas bubbles hold their individuality and do not coalesce during the comparatively extended rise in the pan, especially, the cell walls must stretch rapidly without bursting during the oven spring.

The following factors can be listed as probably being among the most important affecting dough development or dough condition: Mechanical action; water; salts; acids; alcohol, fusel-oil, and other end-products of fermentation; oxidizing agents; phosphatides; enzymes.

The first function of mechanical action in a dough is to incorporate the ingredients and bring together the wetted protein particles so that they cling together. As mixing goes on, the toughness of the dough increases to a maximum and then begins to decrease. The decrease in toughness of an overmixed dough has not been explained, nor can we determine exactly the correct time for mixing except by the method of trial and error.

The stretching of the expanding gas bubbles in a dough probably has a specific mechanical effect, but we cannot define it, nor can we imitate it artificially.

The later mechanical treatment of the dough—punching, rounding, and especially molding—has so important an effect upon its physical condition that two different operators can scarcely obtain identical results. Yet about all that we can say regarding this is that stretching and folding the dough is more satisfactory than indiscriminate wadding together.

The time of standing in contact with water has an important effect on the physical condition of the dough. Measurements of viscosity, plasticity, extensibility, and the like vary widely if the time of standing is altered, and the amount and direction of the variation is different with different flours. Bakers know that with some flours a "dead sponge," in which a part of the flour merely stands in contact with water for a few hours, will give much better volume and texture than

a regular straight-dough method; also that the amount of water used in mixing will materially affect the condition of the dough.

Mineral salts are recognized as having an important effect on dough, particularly the toughening action of calcium salts. A reduction in the hydration of the proteins is the explanation usually offered for this action, but further study is needed.

Acids increase the hydration of gluten, and a decided effect on the physical condition of the dough is easy to observe. With most flours, the addition of acid to the dough noticeably shortens the time of fermentation that will produce the best loaf. During fermentation carbonic, lactic, and acetic acids are produced. The water of the dough will naturally be saturated with carbon dioxide, but the concentration will vary with the atmospheric pressure. Whether this is an important factor in dough conditioning is worthy of experimentation.

The action of alcohol, fusel-oil, and other end-products of fermentation has not been sufficiently studied. It was shown by the author in 1927 that the addition of alcohol in a no-dough-time bread method gave a marked improvement in the resulting bread. A similar improvement was noted recently, using fusel-oil in quantities one-twentieth as great. It is evident that the alcohol and fusel-oil formed during fermentation have an important effect upon dough condition. Here, as with other chemical actions, time of action is almost as important as concentration, as hysteresis, or lag, is a very important characteristic of colloids.

Too little is definitely known of the effect of oxidizing agents upon the quality of dough. The author has found some evidence that at least part of their action is due to changes produced in the phosphatides of the flour. With most flours, oxidizing agents seem to move forward the peak of the curve of dough condition. The fact that fermentation itself is an anaerobic respiration or oxidation, is worth consideration. The addition of phosphatides to certain doughs has been shown to hasten their development, that is, the peak of the curve of dough condition is moved forward. Two trials have indicated a significant increase in the amount of phosphatides dispersed in water in a dough during fermentation. Determinations have not been made on severely overfermented doughs.

Because our knowledge of them is incomplete, enzymes make good scapegoats upon which to place the blame for changes during fermentation. But of those that are best known, only the end-products seem to have an important effect upon dough condition. Further conclusions should await further information.

Thus we see that our dough-condition curve is a composite of many factors acting upon a complicated colloidal mixture. Most of these factors are continuously changing during fermentation. All are so delicately inter-related that the optimum for one factor under a given set of conditions cannot be the optimum when conditions are changed; yet the variables are so many that the number of possible variations is hopelessly large unless we can separate the factors. If the effect of each factor upon a given flour can be accurately determined, it should not be difficult to develop a process of integration that would forecast the correct treatment for obtaining the best possible results.

Practical Applications

It seems apparent that if the best possible volume and texture are to be obtained in bread, the peak of the gas-production curve should approximately coincide with the peak of the dough-condition curve. Further, if a wide fermentation tolerance is to be obtained, a wide portion of the high part of the dough-condition curve must come during the period of adequate gas production.

Adjustment of fermentation methods or of dough ingredients to bring the peak of dough condition into the range of high gas production has long been practiced empirically. If a flour appeared weak, the baker increased the yeast and shortened the fermentation time, thus shortening the time for water and fermentation products to act upon the dough. If the flour appeared too strong, he decreased the yeast and increased the fermentation time, or used a sponge-and-dough method, thus introducing a new peak in the gas-production curve. Perhaps he merely added buttermilk or a highly acid malt extract or a flour improver containing an oxidizing agent, to his original formula. If a satisfactory method was found at once, the flour was all right; if not, he bought a new flour.

But now something more is demanded than such trial-and-error methods. If the baker cannot stay close to the optimum results possible from the ingredients he buys, modern competition will push him out. And it is the cereal chemist who must tell him how to obtain these optimum results. The greatest need of cereal chemistry is fundamental research into the changes taking place during dough fermentation.

Conclusions

The determination of fermentation tolerance as ordinarily carried out is not entirely adequate as a measure of flour quality.

The knowledge obtained will be much more complete if the curve of dough condition is studied separately from that of gas production.

The possibilities of a flour will not be fully understood until the effects of the various factors governing dough condition have been accurately determined for different flours.

Thoro knowledge of the characteristics of a flour will make it easy to adjust fermentation methods and dough ingredients to obtain the best possible bread or the required degree of fermentation tolerance.

COMPARING PROTEIN DETERMINATIONS IN GRAIN WITH THE QUICK METHOD

Preliminary Report from the Analytical Laboratories of the Official Milling Institution at Berlin

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(Received for publication, July 15, 1929)

The treatise of H. Lundin and I. Ellburg from the Central Laboratories, "Quick-Protein-Determination after Kjeldahl,"[1] was the inducement to try this method for the determination of protein in grain and possibly to improve it.

At first, samples of Manitoba and Durum wheats were used. Results were checked by Kjeldahl's ordinary protein determination, with the only difference that phosphorus-sulfuric acid, a mixture of 100 grams phosphorpentoxide, P_2O_5, and 200 grams of concentrated sulphuric acid, H_2SO_4, were used. One-gram samples were used for all determinations.

First Test

The weighed samples were put in Kjeldahl flasks together with 15 cc. of phosphorus-sulfuric acid, one drop of mercury (metallic), and potassium sulphate.

Digestion until colorless required 15 to 17 minutes. After cooling, the closing glass bulbs[2] were cleaned with distilled water and in each case 150 cc. of the water was added. Distillation was carried out by the usual rules with an excess of sodium hydroxide, a little potassium sulfide, and zinc pellets. It took 20 minutes to distill all the

1 Published in "Wochenschrift fuer Brauerei," No. 14 and No. 15, 1929.
2 Glass bulbs are used to partly close the Kjeldahl flasks during digestion.

ammonia. The results showed 2.20% nitrogen for Manitoba wheat flour and 2.04% for Durum wheat flour.

Second Test

One gram of each of the standard test flours was weighed and well suspended with 10 cc. 30% hydrogen peroxide (perhydrol), H_2O_2, and then 8 cc. phosphorus-sulfuric acid was added. This caused a very intensive reaction that resulted in a waterlike, colorless solution. Without supplying heat, 150 cc. distilled water, caustic soda in excess, zinc and potassium sulfide were added and then distilled. The nitrogen content for the Manitoba flour was found to be 1.49% and for the Durum flour 1.31%.

Third Test

The one-gram samples with 10 cc. 30% hydrogen peroxide, H_2O_2 well suspended and 8 cc. phosphorus-sulfuric acid carefully added showed the same intensive reaction (oxygen development) resulting in a transparent, colorless solution. This was heated by a Bunsen burner after adding one drop mercury (metallic). At first the substance turned brown but after boiling for a short time it became whitish. Ten minutes was needed for complete digestion. Distillation was carried out as mentioned above. Manitoba wheat flour showed 2.22% nitrogen and Durum wheat flour 1.99%.

Comparing these three experimental tests, the digestion was found to be unsatisfactory without heating in spite of the fact that hydrogen peroxide and phosphorus-sulfuric acid were used. It was further noticed that the peroxide could not digest the flour in 18 hours without heat being applied to the flask. In fact, the results of the first test and the third test corresponded.

The same tests were repeated with flour from German wheat and castor beans.

Using phosphorus-sulfuric acid and mercury (metallic) the digestion time was checked as 17 minutes for the German flour and 16 minutes for the bean flour. The rapidity of digestion depends very much upon the source of heat. If a good Bunsen burner is used, the substance will be digested in 15 minutes.

Using hydrogen peroxide (perhydrol) the digestion could be made in 12 minutes for the German flour and in 11 minutes for the bean flour. The high moisture in the German flour is supposed to be the cause of the longer time.

It is necessary to heat the substance for digestion even if H_2O_2 has been used. Metallic mercury in connection with H_2O_2 for the reaction has no influence.

Additions of smaller and larger quantities of potassium sulphate, K_2SO_4, as high as 10 grams were tried to shorten the process, but with no success.

In comparison, the application of a mercury-potassium-sulphate mixture, 5 to 10 grams, as used in American laboratories, proved to the best advantage. Tests with German flour and bean flour showed that the addition of 1.5 grams of mercury-potassium-sulphate mixture cut the digestion time 2 to 4 minutes, leaving a transparent solution.

For all following tests one gram of the same flours was used.

1. Employing the method as given under "First Test," 5.75 cc. of 1/5 n hydrochloric acid was needed to neutralize the ammonia from the German flour and 12.35 cc. that from the bean flour, which signifies a protein content of 10.28% and 22.08%.

2. Using H_2O_2 for the determination and heating for 10 minutes showed that 5.85 cc. and 12.40 cc. of 1/5 n HCL were used to neutralize the ammonia. This represents a protein content of 10.45% and 22.35%.

3. The digestion carried out with H_2O_2 and 1.5 grams of the mercury-potassium-sulphate mixture showed that the substance was fully digested after heating for only 2 minutes. For neutralization 5.85 cc. and 12.50 cc. were used. This represents a protein content for the German wheat flour of 10.28% and for the castor bean flour of 22.35%.

A protein distillation generally takes 25 to 30 minutes. These tests showed the same results in 10 minutes if the following method was used.

Add to the digested substance 100 cc. of water, 5 cc. of a 4% potassium sulphide solution, 50 cc. of caustic soda (32%) and distill.

The distillation of the ammonia liberated by the caustic soda required only 10 minutes. Further tests carried out to check these results proved that the distillate going over after boiling 10 minutes did not contain any ammonia. This shortened method for digesting and distilling is no doubt an improvement in the time required to make a protein determination. At any rate, if not employed for the daily routine work, it will be helpful when a quick protein determination is absolutely necessary.

THE SIGNIFICANCE OF ACIDITY IN FLOUR WITH REFERENCE TO FLOUR SPECIFICATIONS

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(Read at the Convention, May, 1929)

The acid-reacting substances in wheat flours and other cereal products have been the subject of a good deal of investigation in late years. It was assumed that the acidity of flour and cereals was an index to the soundness of the flour under certain specified conditions. Not that acidity was a criterion of soundness alone, but an index of a general character. A great difference of opinion exists as to the limits of acidity in a sound flour as well as to the correct methods of making such a determination.

The acidity varies materially with flours of differing quality, depending upon grade or percentage extraction of the flour, and with soundness of the wheat from which it was milled. Briefly stated, the increased acidity of a flour in storage has been assumed to show a gradual hydrolysis of fat to fatty acids, an increase in amino acids, and an increase in phosphoric acid compounds.

A great deal of work on determining the acidity of flour has been carried out in Europe. Planchon, Hilger and Günther, Balland, Lehmann, Kreiss-Aragon, Rammstedt, and others, have proposed various methods of determining the acid-reacting substances in flour. They are all agreed that bacterial and enzymic activity in flour should be inhibited during extraction of the acid-reacting substances, and we find such methods as extracting with alcohol, chloroform, acetone, and even heating quickly to boiling, in water, is advocated. The method used most extensively in Europe is that of Balland, who extracted with 85 per cent alcohol with subsequent titration with standard alkali solution, using tincture of curcuma as an indicator. This method is also known as the Official Greek Method and is used by that government in examining all imported flour. In a subsequent paper Mr. Brooke will describe these several methods, the various limits of acidity allowed by the investigators, and the limits imposed by the Greek government.

Let us now turn attention to our own methods of determining acidity in cereal products. As late as 1907, when the United States Department of Agriculture Bureau of Chemistry Bulletin 107 was published as the Official and Provisional Methods of Analysis of the

Association of Official Agricultural Chemists, no methods were given for analysis of cereal products, that section being in preparation (incidentally, this section was in preparation in the revised edition of Bulletin 107 published in 1912). The first official mention of a method for the determination of acidity in flour of which I have a record, was made by associate referee Ladd, 1909, in his report on cereal products to the A.O.A.C. in 1908. White (1909) reported on the determination of acidity of water extracts of flours by digesting 20 grams of flour for 2 hours with 200 cc. of water at 35°-40° C., titrating 100 cc. aliquots of the filtered extract with 0.05N NaOH, and expressing the results as lactic acid. This differs to some extent from the method suggested by Ladd (1909), in which 18 grams of flour was extracted with water at 40°C. for 10 minutes and then allowed to stand one hour at room temperature, when filtered aliquots were titrated. Ladd mentions that results obtained with flour at temperatures of 15°, 20°, and 25°, respectively, indicate that the acidity increases with the temperature, and the method outlined by him seems to give the maximum acidity.

In 1911, H. L. White (1912) was associate referee of the A.O.A.C. and the Committee on Methods of Cereal Food Analysis suggested for study two alternative methods of determining acidity in flour: (a) Ladd; (b) H. L. White (1909).

The report of the Committee on Cereal Foods, by H. L. White referee in 1913 (1915), recommended a method for acidity in flour essentially a combination of Ladd's and White's methods, as recommended in 1912. The method suggested for study is practically the same as the tentative A.O.A.C. method of today. Eighteen grams of flour was extracted at 40° C. for one or 2 hours, filtered, and titrated with 0.05N NaOH. You will note the time of extraction was one or 2 hours, as the committee could not agree on a specific time of extraction.

The methods for determining the acidity in flour received no further mention in the published proceedings of the A.O.A.C. until 1915, when Committee C, on Recommendation of Referees, Barnard (1917) chairman, recommended the following method for determining acidity, to be published with a view to its adoption as a provisional method in 1916.

> "Weigh out 18 grams of flour into a 500-cc. Erlenmeyer flask and add 200 cc. distilled water free from carbon dioxide. Place flask in water oven kept at a temperature of 40° C. for 2 hours, shaking vigorously every half hour; filter through dry double filters, rejecting the first 10 cc. of filtrate, until 100 cc.

is obtained. Titrate with N/20 sodium hydroxide, using phenolphthalein as an indicator. Each cubic centimeter of sodium hydroxide solution represents 0.050% of acidity in lactic acid."

You will note this method calls for 2 hours digestion, otherwise the method is identical with that published in the A.O.A.C. Book of Methods and designated as only a tentative method.

In 1916, Referee LeClerc (1920) reported a series of collaborative experiments on acidity in flour. Four different methods were used, as follows:

a. Digestion of flour for 2 hours at 40°C.
b. " " " " 1 hour at 40°C.
c. " " " " 10 minutes at 40°C.
and then let stand one hour at room temperature.
d. Digestion of flour for 2 hours at room temperature.

The results obtained by the collaborators showed little differences between the four methods in percentage of acidity obtained, but the significant feature of this report is that the collaborators checked very poorly with each other on the same method.

The chairman approved method C as the simplest, and suggested further study on methods of determining acidity in flour.

No further reference is made to acidity in flour in the published proceedings of the A.O.A.C. from 1917 to 1924; leaving the methods of determining acidity in flour for further study, as recommended in the report of LeClerc. In view of this fact, it is interesting to note that the A.O.A.C. published a tentative method for determining acidity in flour which calls for one hour extraction at 40° C. with which we are all familiar, and is given in their Official and Tentative Methods of Analysis, Edition I (1920) and also in Edition II (1925). This method never was approved or recommended previous to its appearance in Official and Tentative Methods of Analysis so far as can be discerned by our scrutiny of the published proceedings of that association.

In 1925, Referee Hertwig (1926) recommended a simplification in the procedure of determining acidity in flour and further recommended a different basis of reporting results to eliminate the term "lactic acid."

To show how little effort has been made to secure a uniform procedure of determining acidity in flour, our own book of "Methods for the Analysis of Cereals and Cereal Products," sponsored as the approved methods of the American Association of Cereal Chemists, recommends digestion for 10 minutes at 40°C. and then let stand one

hour at room temperature. Another method in common usage consists in digesting for one hour at room temperature. To add to the confusion, we determine the acidity of corn by the method given by Besley and Baston (1914), in which 80% ethyl alcohol is the extractant and results are expressed as the number of cubic centimeters of 0.01N alkali necessary to neutralize the extract for 10 grams of corn.

Your committee on Flour Specifications, consisting of F. A. Collatz, J. T. Flohil, L. R. Olsen, C. H. Bailey, C. C. Fifield, and C. L. Brooke, since its formation a year ago has attempted a definite program of work that dealt with certain restrictions in the acid content of flours imported by Greece. As no uniform method for the determination of acidity was in use throughout the world, American export millers were at a decided disadvantage in determining just what flours could be sold in Greece.

The first problem was a comprehensive review of all the important literature dealing with acidity in flour from both the chemical and the medical standpoints. This literature has been abstracted very completely by Mr. Brooke and will be the basis of his report. This review of the literature was made possible through the financial support of several mills approached by the committee. This shows a fine attitude on the part of the millers and is a plan that might profitably be followed in future co-operative work.

Second, what relationship, if any, exists between results obtained by the Greek official method and those by the tentative A.O.A.C. method as used in this country?

Third, what effect do conditions of storage have upon the rate of increase of the acidity of flours of different grades, and how does this affect their baking qualities? This phase of the work will be reported by Fifield and Bailey in a subreport.

The committee sent out 20 samples of flour for collaborative acidity tests by the Greek method and the A.O.A.C. method. Results are tabulated in Table I. The coefficient of correlation between results of the two methods is $N = 0.255 \pm 0.149$, when the average results of all collaborators for each sample were used in the calculation. This is not a significant correlation of the degree of acidity in the flour, as shown by the two methods. The data indicate very poor agreement between collaborators as to acidity by the tentative A.O.A.C. method, but fairly good agreement by the Greek method. This lack of agreement by the A.O.A.C. method undoubtedly destroys the correlation between the two methods, as the data of Fifield and Bailey, using 71 samples of their own, show a high correlation between the two methods. These correlations are summarized in Table II.

TABLE I

RESULTS OF COLLABORATIVE STUDIES OF ACIDITY IN FLOUR USING GREEK METHOD IN COMPARISON TO A.O.A.C. METHOD

		Collaborator 1		Collaborator 2		Collaborator 3		Collaborator 4		Greek Av.	A.O.A.C. Av.	Ratio
		Greek	A.O.A.C.	Greek	A.O.A.C.	Greek	A.O.A.C.	Greek	A.O.A.C.			
	1	0.093	0.42	0.100	0.390	0.096	....	0.065	0.455	0.096	0.418	1:4.35
		.108	.50	.123	....	.101	....	.115	.445	.112	.447	1:4.00
		.118	.56	.165*	.430	....	....	....	.452	.118	.481	1:4.07
Flour A		.188	.58	.195	.498	.222	0.373*	.155	....	.190	.530	1:2.7
		.196	.58	.204	.510	.230	.495	.211	.635	.210	.555	1:2.64
	2	....	....	.090	.446	.053*	.425	.080	.325*	.085	.435	1:5.10
	3	.152	.56	.154	.570	.158	.475	.152	.490	.154	.524	1:3.4
	4	.118	.56	.108	.540	.148	.485	.115	.515	.114	.525	1:4.6
	5	.127*	.43	.170	.412	.163	.403	.162	.410	.165	.414	1:2.5
	6	.110*	.48	.073	.466	.061	.463	.067	.430	.067	.460	1:6.9
	1	.044	.32	.040	.290	.048	....	.049	.282	.045	.297	1:6.6
		.050	.35	.065	....	.054	....	.060	.290	.057	.320	1:5.6
Flour B		.055	.38	.082	.318	....	....	....	....	....	.350	.....
		.102	.43	.100	.342	.100	.291	.105	....	.102	....	1:3.4
		.210	.52	.211	.442	.231*	.414	.215	.515	.212	.475	1:2.2
	2	....	....	.051	.272	.037*	.248	.055	.235	.053	.252	1:4.7
	3	.073	.35	.075	.336	.092*	.285	.072	.285	.073	.314	1:4.3
	4	.047	.34	.055	.312	.068*	.290	.050	.280	.050	.294	1:6.0
	5	.088	.35	.073	.300	.052*	.320	.075	.325	.079	.332	1:4.2
	6	0.064*	0.30	0.033	0.262	0.033	0.230	0.036	0.270	0.034	0.266	1:7.8

*Omitted from average.

TABLE II

CORRELATION BETWEEN GREEK ACIDITY METHOD AND TENTATIVE A.O.A.C. METHOD ON DIFFERENT GRADES OF FLOUR

Sample No.	Grade of flour	Correlation coefficient Greek method = x; A.O.A.C. = y	
		rxy	
11039	1st Clear durum	0.908±	0.047
11040	2nd Clear spring wheat	.856±	.045
11044	1st Clear durum	.922±	.026
11043	2nd Clear spring wheat	.864±	.044
11330	Patent spring wheat	.734±	.104
All samples (71 Dup.)		0.860±	0.014

Recommendations

The committee has worked on 20 samples of flour during the last year. Two methods of determining acidity have been used—in one the acid-reacting materials were extracted with alcohol and the acidity was determined on the extract. Among the four collaborators this method has, with few exceptions, given fairly concordant results. In the other method an attempt is made to determine the acidity of the flour by extraction with water. Such extracts are complex solutions and we probably not only determine the acid materials already present in the flour but all products of an acid-reacting nature produced by enzymic and bacterial activity. It is assumed that extraction with 85% alcohol does not allow of enzymic or bacterial action, and this method probably gives a better idea of the actual acid-reacting materials in the flour. It is difficult to say at this time which method is preferable; the committee recommends further study of the two methods, with a view to adopting a uniform procedure. This would eliminate a great deal of confusion now existing.

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THE ACIDITY METHOD WITH SPECIAL REFERENCE TO THE ACIDITY LIMITS IMPOSED BY THE GREEK GOVERNMENT

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(Read at the Convention, May, 1929)

Since it is likely that not all of you are familiar with conditions respecting the acidity limits imposed by the Greek government and the extent to which these restrictions have influenced American exports of low-grade flours—particularly spring second clears and durum first clears—I will give a brief summary of the decrees, regulations, protocols, or whatever else they may be termed, issued by the Greek government in the last few years. This includes only the more important aspects of the matter.

Prior to the World War, shipments of wheat and flour from the United States to Greece comprised only an insignificant fraction of the total exports of these commodities. What little flour was imported by Greece was drawn chiefly from European sources. For the period 1910-14 the average annual imports from the United States were only 62,000 bushels of wheat and 1,000 barrels of wheat flour. During and immediately after the War, a considerable trade with Greece in both wheat and flour was built up by American exporters, altho it was not important in relation to the total volume of American trade in these commodities. In 1924, 389,000 barrels of American flour were exported to Greece, or 2.3 per cent of the total wheat flour exports to Europe. In 1925 the wheat flour exports to Greece were 582,000 barrels, or 4.2 per cent of the total. During the following year, 1926, this was reduced to 249,000 barrels, or 2.6 per cent, and in 1927 it amounted to 282,000 barrels, or 2.1 per cent of the total. During the first nine months of 1928 only about 60 per cent as much wheat flour and one-third as much wheat had been exported as during the same period in 1927. The flour trade with Greece is now entirely paralyzed, owing to the combination of these regulations and prohibitive import duties.

The explanation of this marked decrease in wheat flour exports to Greece lies chiefly in the unusual measures adopted by the Greek government, ostensibly for the purpose of protecting the public health.

In 1912 a law was promulgated to standardize the quality of locally milled flour, and also to supply the public with a uniformly milled product. Under this law the maximum acidity of all flour sold in Greece was fixed at 0.250% as sulphuric acid. This limit was sufficiently high to admit practically all low-grade flours of the type used by Greek bakers. Apparently this law was not rigidly enforced, and little was said about the acidity of imported flours until February, 1924, when all flour entering Greece was made subject to chemical examination, the maximum acidity permitted in wheat flour being 0.250% as sulphuric acid, as in the law of 1912.

On December 30, 1924, the Greek Ministry of Health issued a protocol lowering the maximum acidity as sulphuric acid to 0.120% for first quality flour (maximum ash 0.50%) and 0.150% for second quality flour (maximum ash 0.0%). Early in 1925 the limit for second quality flour was restored to 0.250%, only to be lowered several months later to 0.150%. The importation of patent flours was permitted without analysis, provided such flours were accompanied with an official certificate of the exporting country, confirming their quality.

In January, 1926, all flour acidity restrictions were removed, but in April of the same year they were reimposed. In August, 1926, the maximum acidity of second quality flour was increased from 0.150% to 0.160%, with a tolerance of 10% of the maximum during June, July, and August. No further changes in acidity regulations have been made.

In defense of these drastic restrictions on the acidity of imported flour, the Greek Ministry of Health claims that flour containing more than the maximum allowed acidity is unfit for human consumption. They claim that a certain French chemist has stated that flour containing more than 0.150% acidity as sulphuric acid is prejudicial to the health. As stated in an editorial in the Northwestern Miller for April 1, 1925, "it is not apparent that the proposal of the Greek chemists [to lower the acidity to 0.150%] is based on any real concern for the purity and wholesomeness of imported flour, for there is no record of any complaint on other than purely technical grounds against flour imported from America. This leaves strong reason to believe that the question is more political than hygienic or economic, and that the real purpose of imposing special tests on imported flour is in the interest of the Greek millers."

On June 17, 1925, a bulletin was sent out to its members by the Millers National Federation, containing information received by the

Department of Commerce regarding the regulations covering the acidity of flours imported into Greece. According to this report, the Greek limit of 0.150% acidity as sulphuric acid is equivalent to 0.275% as lactic acid. This figure obviously was obtained by multiplying 0.150 by 1.83, the factor for converting sulphuric acid into lactic, without taking cognizance of a possible difference in the methods used to determine acidity. It was claimed in this bulletin that almost all first clears would fall within this limit, altho certain second clears would exceed it. American exporters merely needed to make sure that their flours fell somewhat below 0.150% as sulphuric or 0.275% as lactic acid.

Exception was taken to this statement by a Greek correspondent of the Northwestern Miller who was in close touch with the situation from the flour importers' point of view. This correspondent stated that altho it was true that a few shipments of clears had given an acidity of 0.130 to 0.140% when analyzed in Greece, it was none the less a fact that the greater part of the shipments of clear flours were giving an acidity exceeding the 0.150 point. An extract was cited from a letter received from one of the largest American milling companies, which stated that freshly milled durum first clear tested 0.161% acidity as sulphuric at the mill, and freshly milled hard wheat second clear tested 0.214%. A superior quality of hard wheat first clear tested 0.124% acidity as sulphuric acid.

Last year the Committee on Flour Specifications of the A.A.C.C. began a thoro study of the acidity of flour as determined by the official method and by the Greek official method. An important phase of this work was a search of all available literature for information on methods and especially on the possible harmful effects of high acidity on the health. In other words, it was desired to find out if there was any scientific basis for the statement of the Greek Ministry of Health and Hygiene that flour containing more than 0.150% acidity as sulphuric acid was injurious to the health.

In my search of the literature for information on the above points, I consulted a great many journals and texts in English, French, German, the Scandinavian languages, and Italian. As for the physiological aspects of the question, I may state briefly that I found nothing to substantiate the claims of the Greek authorities, nor did I find any record of acidity restrictions in any other country. The acidity limits set by different investigators vary so widely as to indicate that the effect of acidity on health, if considered at all, is of slight importance.

The method now used in Greece as the official method for

testing imported flours is so similar to the method which, so far as we know, originated with the French chemist Balland, that it probably would be safe to refer to the Greek method as the Balland method. Balland published work on the acidity of flour as early as 1883, and apparently used the alcohol extraction method from the beginning of his work, altho the details of his method did not appear until 1894. Practically all the European investigators who have published work on acidity as determined in an alcoholic extract, report the use of the same method. This gave a valuable lead in the review of the literature, as it appeared likely that if the Greeks had borrowed Balland's method, they also had borrowed some of his ideas regarding the acidity limits in flour.

Dr. Collatz has already reviewed the development of the A.O.A.C. method for the determination of acidity, and has explained how it came to be adopted. A number of aqueous extraction methods are used in Europe. A good deal of discussion has appeared in the literature regarding the relative merits of the aqueous and alcoholic extraction methods, the most frequent objection to the aqueous extraction being that enzymic action is set up in the extract and that consequently the acidity thus determined does not represent the true or original acidity of the flour. A further objection is that the color change with phenolphthalein is not so sharp in water as in alcohol. It was maintained by Rammstedt, in 1913, that in flour from sprouted wheat the acidity is far greater in aqueous extract than in alcoholic, because of enzymic action. This was also true of flours known to contain acid-producing bacteria. Rammstedt concludes that extraction with boiling alcohol for 30 minutes and titration of the filtered extract gives unexceptional results, as it shows only the original acidity, while extraction with boiling water and titration of the resulting suspension gives a much higher value because of the action of bacteria and enzymes in aqueous solution. Consequently, if it is desired to learn the original acidity of a flour, the alcoholic process is recommended. If it is a question of determining the behavior of a flour during fermentation, or of studying its enzymatic properties, the boiling water method should be used. Such studies might contribute greatly, Rammstedt says, toward solving various problems in the chemistry of flour and bread.

Rammstedt's conclusion that the alcoholic extraction method is the only one that gives satisfactory results was hotly disputed by Kreis and Arragon, authors of the method in which the acidity is determined

by boiling the flour with water for 30 minutes and titrating the suspension. These authors claim that the enzymic action taking place during the period required for the flour-water mixture to reach a temperature high enough to inhibit such action is not perceptible. To prove this, a series of flours were first treated with alcohol to inhibit enzyme activity, and then boiled with water for five minutes. The results obtained checked closely with those obtained without the preliminary alcohol treatment. Other experiments of the same nature show, they believe, that the hypothesis of acid formation through enzymic action or bacteria is ungrounded. These authors also found that if a flour was extracted:

A—with boiling water for 30 minutes and the suspension titrated;
B—with boiling alcohol for 30 minutes and the filtrate titrated;
C—the acidity from the alcohol extract determined by boiling with water 30 minutes and the suspension titrated,

the acidity of the filtrate from the alcoholic extract added to the acidity of the boiled-in-water suspension of the residue from the alcoholic extract was equal to the acidity of the original sample determined by boiling in water 30 minutes and titrating the residue.

Thus it seems that the acid-reacting constituents of flour are largely insoluble in alcohol. Kreis and Arragon regard all methods in which acidity is determined on the alcoholic extract as essentially erroneous. In their opinion sound flour contains not free acids but acid salts, probably phosphates, which are insoluble in alcohol.

The method of extraction through boiling with water, however, gives far higher results than any of the other methods commonly used in Europe. In a comparative study made by Kalning, in 1919, the Kreis-Arragon method gave higher results than any of the dozen-odd methods employed, being, for instance, three times as high as the Balland method of extraction for 24 hours with alcohol. It is true that in the Kreis-Arragon method the acidity is determined on the suspension and not the filtrate. When one takes into consideration that on the average the acidity of the filtrate is about half the acidity of the suspension, it is obvious that the boiling water method gives results not greatly in excess of those obtained by the usual methods of extraction at low temperatures.

I might add that good results have been obtained through the use of acetone as an extraction medium. This is especially true when bread or dough is to be examined, as the acetone breaks up this material very effectively.

TABLE I

SULFURIC ACID ACIDITY OF ALCOHOLIC AND AQUEOUS EXTRACTS USING CURCUMA AND PHENOLPHTHALEIN AS INDICATORS

Extraction time, days		1	3	6	7	9	11	13	16	17	19	Increase
High-Grade Flour												
90 % Alc.	Curcuma	0.026	0.026	0.027	0.031	0.034	0.028	0.030	0.030	0.030	0.028	0.008
	Phthn.	0.023	0.025	0.027	0.030	0.032	0.032	0.032	0.032	0.039	0.041	0.016
Water	Curcuma	0.094	1.04	1.59	2.02	1.76	2.24	2.47	2.25	2.21	2.33	2.38
	Phthn.	0.083	1.16	1.63	2.02	1.76	2.17	2.32	2.17	2.08	2.21	2.24
Low-Grade Flour												
90 % Alc.	Curcuma	0.037	0.039	0.033	0.064	0.056						0.031
	Phthn.	0.047	0.044	0.047	0.064	0.078						0.034
Water	Curcuma	0.151	1.69	2.77	2.88	2.40						2.73
	Phthn.	0.151	1.71	2.72	2.85	2.30						2.73
8-Year-Old Patent Flour												
90 % Alc.	Curcuma	0.093	0.113	0.123	0.111	0.123						0.030
	Phthn.	0.102	0.123	0.127	0.115	0.134						0.032
Water	Curcuma	0.151	0.353	1.262	1.642	1.666						1.51
	Phthn.	0.126	0.353	1.262	1.541	1.666						1.54

You have heard something about curcuma as an indicator. Mr. Fifield and others who have collaborated in the acidity studies will testify, I think, that curcuma is a better indicator than it at first appeared to be. A comparison between phenolphthalein and curcuma in both aqueous and alcoholic extracts is included in Table I, which also shows the increase in acidity with time. This work was done by Arpin and Pecaud, French investigators.

Time does not allow a discussion of all the interesting methods used for the determination of acidity. I had considered preparing a large chart giving the details of all the methods found, as well as the limits stated in each case. This is complicated by differences in methods and terminology. Table II gives limits set by a number of investigators using Balland's method, or at any rate a closely similar method, as well as limits set by two investigators using aqueous extraction methods. It will give you some idea of the diversity in opinion regarding the safe limits for acidity in flour.

TABLE II

ACIDITY LIMITS

	Alcohol extraction	
Reported by	Description of sample	Acidity as sulfuric acid
Balland	Normal flour	0.015-0.040
Planchon	Sound flours	.030- .133
	Damaged flour	.160
Marion and Manget	Spoiled flours	.120-Soft
		.070-Hard
Vauflart	Fresh flour	.020
	Spoiled flour May rise to	.120
	Sound—good gluten	.027- .046
	Old—poor gluten	0.085-0.183
	Baking quality not impaired if acidity does not exceed 0.100	
	Water extraction	Acidity as lactic acid
White	Patents, 1908 crop	0.108-0.300
	Patents, 1909 crop	.114- .260
	Straights, 1909 crop	.103-0.297
Swanson, et al.	Short patents	.128
	Long patents	.163
	Straights	0.166

I have also collected some of the data showing the ratio between the acidities of flour when determined with alcohol and with water as extraction media.

Rammstedt found that extraction with water for 12 hours at 45-50° gave about three times as high results as extraction with boiling alcohol for 30 minutes.

Planchon found that extraction with water for 24 hours and with alcohol for the same period gave acidities having a ratio of 1.42 : 1.

Kalning determined the acidity on two flours by a variety of methods. In this series of experiments no definite relation appeared between the acidities as determined in alcohol or other fat solvents and in water. Extraction with water at room temperature for an hour and titration of the filtrate with phenolphthalein gave almost exactly the same result as extraction with absolute alcohol in a Soxhlet apparatus for 12 hours and titration of the filtrate with litmus, extraction with chloroform for 14 hours and titration of filtrate, boiling with absolute alcohol 30 minutes and titration of filtrate with phenolphthalein, and gave a figure almost twice as high as obtained by extraction with 85-90% alcohol for 12 hours and titration of filtrate with curcuma (Balland's method).

The methods commonly used for the determination of acidity are characterized by two general trends. In one the acid-reacting organic constituents are extracted with alcohol and the acidity is determined on the extract. Most of the methods proceed along these lines. In the other group an attempt is made to determine the total acidity of the constituents formed by phosphorus compounds. In this case the solvent is water. Since the question of the nature of the acids of flour is not yet sufficiently clear, it is difficult to say which process is to be preferred. It is desirable that a uniform method be devised for acidity or at least that the method employed be specified in reporting analytical results. This would eliminate a great deal of misunderstanding.

With regard to the significance of acidity of flour in relation to health, a few extracts from my abstracts may be of interest. Most of these refer to the acidity of bread, not of flour. Obviously it is possible to make a highly acid bread from a flour of low acidity.

Lehmann, a German investigator, stated in 1894 that in a diet including the average amount of meat, bread of high acidity was useful in effecting a better assimilation of the meat. He found that in some cases acid bread alone was well assimilated by healthy persons.

Lehmann also found that high acidity was useful in rendering harmless various toxic fungi, such as ergot.

Balland wrote in 1886 that in his opinion poisonous alkaloids developed in flour after it had stood in sacks a considerable time. He obtained in old flours a reaction which he claimed indicated the presence of toxic substances.

In the Journal of the American Medical Association, in 1922, an editorial stated:

> "The impression that foods which have an unmistakable acidity act as real acids is still widespread among the laity, and also finds occasional expression on the part of physicians. Modern biochemical investigation has clearly demonstrated, however, that the natural reaction of a food is no criterion of the part it may play as acid or base in the metabolism. The processes by which energy is liberated are essentially oxidative in character. The ingesta are normally subjected to oxidation in the body. Consequently it may readily happen that a food which is acid in reaction may from the standpoint of metabolism, be essentially basic."

Mohs showed in 1928 that spoiled wheat in which the proteins were broken down contained substances that were toxic to yeast, which he terms toxamines. These are especially plentiful in musty and bitter-tasting flours, showing that toxamines are formed more through internal decomposition and mold than through the action of acid-forming bacteria.

Saundby, in an article in the Lancet in 1927, called attention to a French nutrition expert's claim that bread may be a source of uric acid through setting free an excess of phosphoric acid.

Obviously, none of these have a direct bearing on the problem of the acidity of flour. I cite them because they are practically the only references I found that might be used in formulating objections to high acidity in otherwise sound flour.

THE MARCH OF ACIDITY IN STORED FLOURS[1]

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(Read at the convention, May, 1929)

It has been generally observed that the acidity of flour increases progressively with the lapse of time. Certain of the earlier investigations that support this assumption have been reviewed by Bailey (1925, particularly pages 182 to 189). The rate of change in acidity has appeared to be a function of flour grade, moisture content, humidity of the atmosphere, temperature, and possibly other factors. This increase in acidity in stored flours has become a factor of substantial importance to American millers since the Greek Government has imposed limits of acidity on flour imported into that country.

The Greek Government limits of 0.15 per cent acidity, as determined by a method prescribed by government chemists of that country, has resulted in this investigation, which was designed to disclose the time that must elapse for flours of various grades and stored under various conditions to reach or exceed this limit. A history of the situation respecting the imposition of these acidity limits has been traced in the paper by C. L. Brooke.

According to the best information available to us at this time, the following method has been used by Greek chemists in the determination of the acidity of flour to be examined under their statutes:

> Weigh 5 grams of flour into an 80 cc. flask provided with a ground glass stopper and cover with 25 cc. of 85 per cent alcohol. Allow to digest for 24 hours with frequent agitation. Draw off 10 cc. of the supernatant liquid with a pipette and titrate with alcoholic 0.02 N NaOH using tincture of curcuma as an indicator. The end point is a persistent "chamois" color. Each cc. of the standard alkali is equivalent to 0.00098 grams of H_2SO_4.

In addition to the examination of flour by this procedure, acidity was determined by the A.O.A.C. (1925) tentative method, also the H-ion concentration and the percentage of nitrogen peptized by 5% K_2SO_4 solution. It was thought that possibly the last-named determination would disclose any substantial decomposition of the native proteins of the flour.

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Each flour used for these storage experiments was divided into four portions, which were maintained under different storage conditions. Three of these portions were placed in air thermostats maintained at 25°, 30°, and 35°C. respectively, with the relative humidity of each cabinet maintained at 80± per cent. The fourth sample was placed in a large refrigerator, which was maintained at 3° to 4° C. All four portions were contained in ordinary cotton sacks.

During the course of the experiments five samples of commercial flour were used:

1. A patent flour (Lab. No. 11330) containing 10.9% of crude protein, and 0.43% of ash.
2. A first clear durum wheat flour (Lab. No. 11039) containing 11.95% of crude protein and 1.07% of ash.
3. A first clear durum wheat flour (Lab. No. 11044) containing 11.85% of crude protein and 1.03% of ash.
4. A second clear spring wheat flour (Lab. No. 11040) containing 14.31% of crude protein and 1.68% of ash.
5. A second clear spring wheat flour (Lab. No. 11043) containing 15.21% of crude protein and 1.75% of ash.

Observations Upon Durum First Clear Flour

The first clear durum wheat flours contained 0.057 and 0.050% of acidity (Greek method) about a week after they were milled. The flour stored at the lowest temperature, 3°C., showed the lowest rate of increase in acidity of any of the portions of the sample. The rate of change was manifestly a function of temperature, as evidenced by the data recorded graphically in Figure 1 for flour No. 11044. Both samples of first clear durum wheat flour behaved in a similar manner, and the data recorded graphically for the one sample are typical for this grade. In fact, if the corresponding data for sample No. 11039 were included in this series of graphs, the value would nearly superimpose upon the data for sample 11044. The detailed data from the studies of both samples are recorded in Table I.

The rate of increase in acidity appears to diminish with the lapse of time, altho it was at a fairly high level throughout this experiment, which extended over 190 days. In the sample stored at 35°C., the Greek limit of 0.15% acidity was reached about the 90th day, while in the sample stored at 30° and 25° it required the equivalent of about 110 and 140 days, respectively, to reach this limit. The sample maintained in cold storage never quite reached the maximum limit of 0.15% during the 190 days that it was under observation.

TABLE I

ACIDITY AND OTHER CHEMICAL DETERMINATIONS RECORDED DURING THE STORAGE OF DURUM FIRST CLEAR FLOURS

	Laboratory No. 11039				
Date sampled Days after milling	11/11/28 11	11/23/28 23	12/26/28 57	2/18/29 110	5/21/29 202
Stored at 3°C.					
Greek acidity %	0.057	0.057	0.065	0.087	0.134
A.O.A.C. acidity %	0.252	0.265	0.280	0.358	0.390
Moisture %	13.24	13.64	14.00	14.22	13.20
pH	6.40	6.45	6.49	6.35	6.40
N in 5% K_2SO_4 sol. %	0.23	0.23	0.24	0.24	0.23
Stored at 25°C.					
Greek acidity %	0.057	0.062	0.082	0.120	0.167
A.O.A.C. acidity %	0.252	0.270	0.295	0.400	0.420
Moisture %	13.24	8.06	9.74	10.16	9.30
pH	6.40	6.45	6.47	6.32	6.38
N in 5% K_2SO_4 sol. %	0.23	0.24	0.24	0.24	0.24
Stored at 30°C.					
Greek acidity %	0.057	0.066	0.084	0.134	0.197
A.O.A.C. acidity %	0.252	0.315	0.325	0.405	0.430
Moisture %	13.24	7.40	9.02	11.02	9.23
pH	6.40	6.47	6.47	6.30	6.31
N in 5% K_2SO_4 sol. %	0.23	0.25	0.25	0.25	0.23
Stored at 35°C.					
Greek acidity %	0.057	0.062	0.091	0.154	0.243
A.O.A.C. acidity %	0.252	0.320	0.330	0.410	0.460
Moisture %	13.24	6.24	9.72	12.22	10.23
pH	6.40	6.47	6.47	6.31	6.24
N in 5% K_2SO_4 sol. %	0.23	0.25	0.24	0.24	0.23

	Laboratory No. 11044				
Date sampled Days after milling	11/21/28 8	12/12/28 30	1/18/29 64	3/18/29 124	5/23/29 190
Stored at 3°C.					
Greek acidity %	0.050	0.076	0.082	0.105	0.142
A.O.A.C. acidity %	0.280	0.295	0.320	0.330	0.322
Moisture %	11.28	13.44	13.52	13.40	13.66
pH	6.57	6.49	6.54	6.42	6.32
N in 5% K_2SO_4 sol. %	0.21	0.24	0.27	0.25	0.28
Stored at 25° C.					
Greek acidity %	0.050	0.082	0.096	0.128	0.174
A.O.A.C. acidity %	0.280	0.302	0.325	0.345	0.390
Moisture %	11.38	9.76	9.72	10.20	9.80
pH	6.57	6.47	6.49	6.37	6.37
N in 5% K_2SO_4 sol. %	0.21	0.25	0.25	0.27	0.28
Stored at 30°C					
Greek acidity %	0.050	0.080	0.103	0.142	0.192
A.O.A.C. acidity %	0.280	0.300	0.340	0.355	0.430
Moisture %	11.38	8.46	9.84	10.63	9.00
pH	6.57	6.49	6.45	6.37	6.32
N in 5% K_2SO_4 sol. %	0.21	0.24	0.26	0.26	0.30
Stored at 35°C.					
Greek acidity %	0.050	0.076	0.118	0.165	0.227
A.O.A.C. acidity %	0.280	0.315	0.350	0.360	0.465
Moisture %	11.38	7.78	10.48	11.86	10.30
pH	6.57	6.47	6.43	6.29	6.20
N in 5% K_2SO_4 sol. %	0.21	0.24	0.27	0.24	0.30

In the same chart the data resulting from the determination of acidity by the A.O.A.C. tentative method are recorded graphically in the four lower graphs. These values are higher in terms of percentage than are those obtained by the Greek method, doubtless because of the radical difference in the methods of extraction.

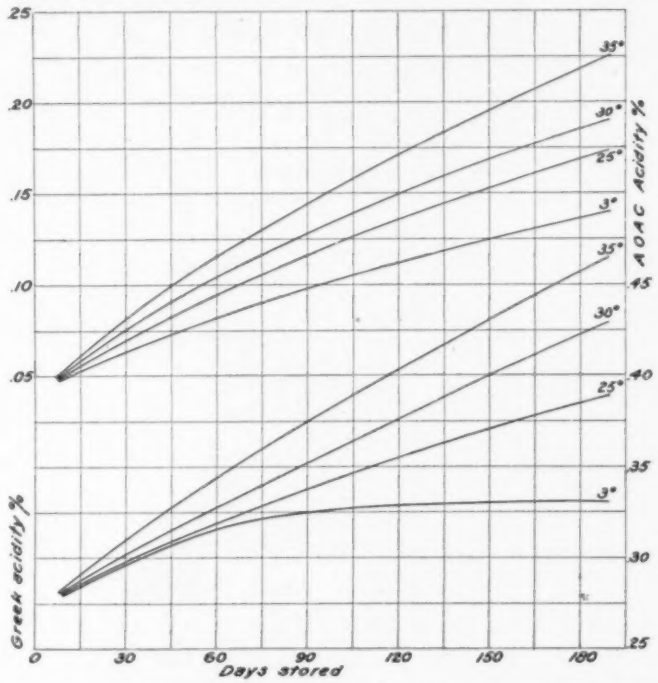

Fig. 1. Change in Acidity of Durum First Clear Flours with the Lapse of Time when Stored at Different Temperatures

The four upper graphs represent acidity as determined by the Greek method; the four lower graphs, acidity as determined by the A.O.A.C. method, for Sample 11044.

In the application of the A.O.A.C. tentative method, the flour is extracted with water at 40° for 60 minutes. The acidity of the resulting extract is probably the sum of acid-reacting materials (or more properly alkali-neutralizing materials) present in the original flour, plus alkali-neutralizing substances produced during the extraction. At the temperature employed and in the presence of water, it seems probable that enzymic phenomena may affect the production of a considerable portion of the total acidity, so the determination really measures the capacity of the flour to produce acids as well as the initial concentration of acids before the extraction medium is applied. The direct comparison of data secured by the two methods is further complicated by the difference in solvents.

Certain acid-reacting materials may be extracted with water that are not removed by 85% alcohol, and vice versa. The fact that results are expressed as lactic acid in the A.O.A.C. method and as sulfuric acid in the Greek acidity method would not introduce serious complications were it not for the radical differences between the two methods, as otherwise one set of values could be calculated in the terms of the other by the application of a suitable factor.

We are not in a position to suggest a factor for the conversion of determinations by the Greek method into terms of determinations by the A.O.A.C. method without recourse to empirical procedures. Using data afforded by the collaborative studies conducted under the direction of F. A. Collatz, the approximate ratios widen with decreasing acidity by either method. A rough calculation, based upon 80 comparisons or pairs of determinations, has given the values in the following table. It appears that the difference is greater at the lower levels of acidity than at the higher levels, and accordingly, as the acidity increases a smaller factor must be used in multiplying the percentage of acidity as determined by the Greek method to convert it into terms of acidity as determined by the A.O.A.C. method.

Acidity percentage by Greek method	Multiply by this factor to convert into acidity by A.O.A.C. method
0.05	6.0
0.10	4.2
0.15	3.3
0.20	2.4

At the time that the determinations of titratable acidity were made, the H-ion concentration of the flour was determined and recorded as pH. The changes in pH were slow, owing, no doubt, to the high buffer index of the samples. It accordingly appears that the H-ion concentration is less useful in tracing changes in acidity with the lapse of time than the percentage of titratable acidity.

The changes in the percentage of nitrogen in compounds peptized by 5% K_2SO_4 solution were of relatively small magnitude. In sample 11039 the differences between the freshly milled flour and that stored for 202 days were within the limits of the experimental error of the method, while in sample 11044 the greatest increase was equivalent to about 0.09 per cent in terms of nitrogen.

It seems inaccurate to characterize flours as either sound or unsound on the basis of the percentage of acidity alone, and the authors are under the impression that the majority of American cereal chemists do not regularly use flour acidity as a measure of soundness. It is probable that certain biological situations might result in the development

of a measure of unsoundness in flour without resulting in any increase in acidity, and that a normal and progressive increase in acidity might result in many instances without involving what would properly be termed unsoundness.

As indicated in the foregoing paragraph, we could detect no definite evidence of protein decomposition as indicated by the quantity of nitrogen in the compounds peptized by 5% K_2SO_4 solution. To afford further evidence of the degree of soundness of these flours, they were subjected to experimental baking tests. After more than six months of storage, sample No. 11039 baked into bread of volume, texture, and other qualities equal to those of the fresh flour, and the bread was eaten with relish by observers in the laboratory. Neither flavor, odor, nor physical properties merited any assumption that the flours at this time were in any sense unsound, even tho their acidity had in certain instances reached a level equivalent to more than 0.20% as determined by the Greek method.

Observations Upon Spring Wheat Second Clear Flour

The two samples of spring wheat second clear flour (Lab. Nos. 11040 and 11043) were aliquoted and stored under the same conditions as were maintained in storing the durum first clear flours. These two flours contained 0.093 and 0.115% of acidity (Greek method) about a week after they were milled. The data resulting from the determination of acidity by the two methods are recorded in Table II and graphically in Figure 2 for sample 11040. The rate of increase in acidity in this sample was somewhat lower than in the second lot of the same grade of flour (Lab. No. 11043), so the values here recorded are probably conservative rather than otherwise so far as they represent change in acidity on storage of flours of this grade.

As with the durum first clear flour, the rate of increase in acidity was greatest at the beginning of the experiment and diminished with the lapse of time. This was particularly true in the values resulting from the application of the A.O.A.C. method, where a very slow rate of change was evident after the lapse of about 135 days. Again, the rate of increase in acidity as determined by both methods was a function of temperature, being at a progressively higher level as the temperature was increased from 3° to 35°C.

TABLE II

ACIDITY AND OTHER CHEMICAL DETERMINATIONS RECORDED DURING THE STORAGE OF SPRING WHEAT SECOND CLEAR FLOUR

	Laboratory No. 11040				
Date sampled Days after milling	11/11/28 11	11/23/28 23	12/26/28 57	2/18/29 110	5/21/29 202
Stored at 3°C.					
Greek acidity %	0.092	0.114	0.154	0.223	0.322
A.O.A.C. acidity %	0.442	0.440	0.510	0.575	0.590
Moisture %	12.54	12.54	13.58	13.80	12.30
pH	6.43	6.44	6.44	6.21	6.31
N in 5% K_2SO_4 sol. %	0.35	0.38	0.34	0.32	0.31
Stored at 25°C.					
Greek acidity %	0.092	0.130	0.194	0.260	0.344
A.O.A.C. acidity %	0.442	0.445	0.500	0.605	0.635
Moisture %	12.54	8.05	9.56	10.00	9.10
pH	6.43	6.44	6.41	6.24	6.34
N in 5% K_2SO_4 sol. %	0.35	0.37	0.34	0.32	0.32
Stored at 30°C.					
Greek acidity %	0.092	0.129	0.192	0.277	0.369
A.O.A.C. acidity %	0.442	0.450	0.500	0.620	0.650
Moisture %	12.54	6.98	8.88	11.04	9.00
pH	6.43	6.43	6.41	6.24	6.29
N in 5% K_2SO_4 sol. %	0.35	0.38	0.34	0.32	0.31
Stored at 35°C.					
Greek acidity %	0.092	0.124	0.204	0.306	0.436
A.O.A.C. acidity %	0.442	0.490	0.510	0.650	0.680
Moisture %	12.54	5.76	8.34	11.90	10.76
pH	6.43	6.47	6.41	6.25	6.05
N in 5% K_2SO_4 sol. %	0.35	0.39	0.35	0.32	0.30
	Laboratory No. 11043				
Date sampled Days after milling	11/21/28 8	12/12/28 30	1/18/29 64	3/18/29 124	5/23/29 190
Stored at 3°C.					
Greek acidity %	0.115	0.172	0.208	0.282	0.320
A.O.A.C. acidity %	0.515	0.550	0.560	0.610	0.675
Moisture %	12.04	13.64	13.40	13.30	13.90
pH	6.53	6.35	6.47	6.39	6.26
N in 5% K_2SO_4 sol. %	0.39	0.39	0.38	0.41	0.40
Stored at 25°C.					
Greek acidity %	0.115	0.195	0.255	0.312	0.374
A.O.A.C. acidity %	0.515	0.600	0.640	0.650	0.720
Moisture %	12.04	9.66	9.50	10.20	9.20
pH	6.53	6.45	6.45	6.37	6.36
N in 5% K_2SO_4 sol. %	0.39	0.39	0.39	0.42	0.42
Stored at 30°C.					
Greek acidity %	0.115	0.195	0.270	0.347	0.387
A.O.A.C. acidity %	0.515	0.605	0.650	0.660	0.750
Moisture %	12.04	8.12	9.38	10.86	9.40
pH	6.53	6.44	6.44	6.39	6.35
N in 5% K_2SO_4 sol. %	0.39	0.39	0.38	0.42	0.40
Stored at 35°C.					
Greek acidity %	0.115	0.181	0.284	0.384	0.500
A.O.A.C. acidity %	0.515	0.560	0.615	0.635	0.800
Moisture %	12.04	7.66	10.20	11.83	9.70
pH	6.53	6.45	6.46	6.22	6.14
N in 5% K_2SO_4 sol. %	0.39	0.39	0.38	0.41	0.39

The evidence of protein cleavage, as revealed by the percentage of nitrogen in the extracts with 5% K_2SO_4 solution, is even more definitely lacking in the samples of spring wheat second clear flour than in the durum first clear flour. The differences in this property were within the limits of experimental error except in a few cases in which the percentage decreased rather than increased. The quantity of nitrogen and the protein so peptized was greater in the second clear flours than in the first clear, as is usual when such comparisons are made.

Both these samples of spring wheat second clear flour were baked on November 21, 1928, three weeks after sample No. 11040 was milled, and one week after sample 11043 was milled. They were baked at intervals during the next few months, the last baking test being conducted on June 28, 1929. In both instances the loaves baked on the last date were superior in quality to those baked the preceding November, more than seven months earlier, as judged by the higher scores assigned in the experimental bakery, except for the flour stored at 35°C. In this instance the decrease in score was relatively small, especially when the extreme conditions of storage at this high temperature are considered. The bread had no flavor or other quality that would characterize it as baked from unsound flour.

Figure 2 indicates that flour No. 11040 contained more than 0.15% acidity (Greek method) after 40 days of storage at 25°, 30° and 35°C. The samples stored in the refrigerator at 3°C. reached this limit of acidity in approximately 50 days. In sample No. 11043 the rate of increase in acidity was even greater, as has already been mentioned, and all the samples contained more than 0.15% before 30 days of storage, including the sample in cold storage at 3°C.

A third sample of the same grade of flour was obtained on March 1, 1929, immediately after milling, and was held in storage for 82 days. Its composition at the end of this period showed that the rate of increase in acidity was even greater than that of sample 11040 (Fig. 1). Interpolations of the data obtained with sample 11290 indicate that the three portions maintained at a temperature of 25° or above contained more than 0.15% acidity (Greek method) at the end of 40 days or less of storage.

Observations on the Change of Acidity in Patent Flour

One sample of patent flour was used in these investigations, chiefly in order to afford a fairly complete series of flours representing different grades, altho high grade flours of this sort are unlikely to develop a degree of acidity high enough to exceed the limits under discussion.

The acidity data resulting from the periodic analyses of these samples are recorded graphically in Figure 3, and it will be observed that after more than 150 days of storage none of the samples contained the equivalent of 0.15% of acidity. We are inclined to believe that if the limit of 0.15% of acidity had been set up as applied to straight grade and patent flours containing less than 0.50% of ash, the limit might be a rational one, as our experience indicates that there must be some very radical changes in such flour before this limit of acidity is exceeded.

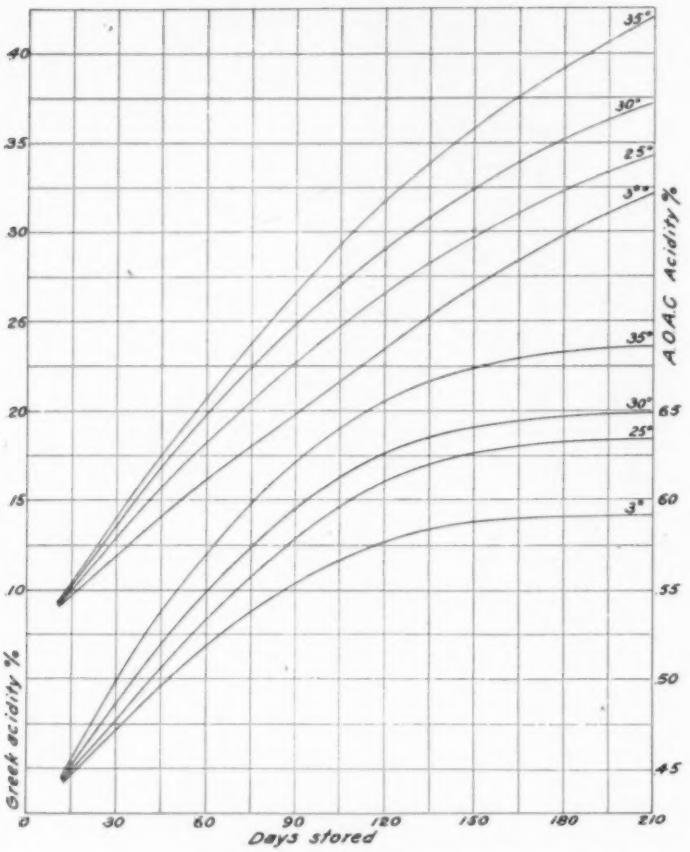

Fig. 2. Change in Acidity of Spring Wheat Second Clear Flours with the Lapse of Time when Stored at Different Temperatures
The four upper graphs represent acidity as determined by the Greek method; the four lower graphs, acidity as determined by the A.O.A.C. method, for Sample 11040.

Table III indicates that the changes in percentage of nitrogen and the protein peptized by 5% K_2SO_4 solution were small, and are probably within the limits of experimental error, altho the differences, if any, are in the direction of decrease rather than increase.

TABLE III

ACIDITY AND OTHER CHEMICAL DETERMINATIONS RECORDED DURING THE STORAGE OF PATENT FLOUR

	Laboratory No. 11330				
Date sampled	1/4/29	1/18/29	3/18/29	5/21/29	6/3/29
Days after milling	6	20	80	143	156
Stored at 3°C.					
Greek acidity %	0.040	0.040	0.050	0.076	0.077
A.O.A.C. acidity %	0.105	0.110	0.115	0.160	0.165
Moisture %	14.00	13.72	13.73	13.10	12.30
pH	6.39	6.35	6.39	6.28	6.30
N in 5% K_2SO_4 sol. %	0.15	0.16	0.15	0.15	0.14
Stored at 25°C.					
Greek acidity %	0.040	0.040	0.065	0.079	0.097
A.O.A.C. acidity %	0.105	0.120	0.140	0.175	0.168
Moisture %	14.00	10.56	10.66	10.00	10.60
pH	6.39	6.31	6.32	6.26	6.24
N in 5% K_2SO_4 sol. %	0.15	0.16	0.15	0.15	0.14
Stored at 30°C.					
Greek acidity %	0.040	0.045	0.075	0.089	0.105
A.O.A.C. acidity %	0.105	0.130	0.145	0.175	0.190
Moisture %	14.00	11.20	11.70	9.95	10.10
pH	6.39	6.31	6.31	6.24	6.22
N in 5% K_2SO_4 sol. %	0.15	0.16	0.16	0.15	0.14
Stored at 35°C.					
Greek acidity %	0.040	0.049	0.082	0.109	0.117
A.O.A.C. acidity %	0.105	0.150	0.153	0.185	0.195
Moisture %	14.00	11.90	12.83	10.10	11.26
pH	6.39	6.32	6.25	6.12	6.10
N in 5% K_2SO_4 sol. %	0.15	0.15	0.16	0.14	0.13

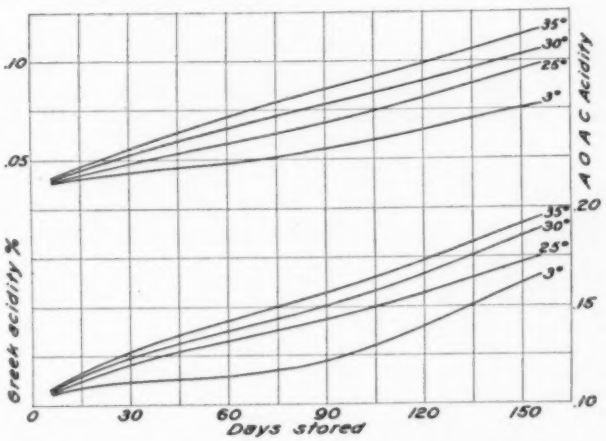

Fig. 3. Change in Acidity of Patent Flour with the Lapse of Time when Stored at Different Temperatures

The four upper graphs represent acidity as determined by the Greek method; the four lower graphs, acidity as determined by the A.O.A.C. method, for Sample 11330.

Conclusions

Spring wheat second clear flour that contained about 0.1±% of acidity (Greek method) when freshly milled increased in acidity at a rapid rate under good storage conditions. After 40 days or less, at temperatures from 25° to 35°C. the acidity exceeded the limit of 0.15% imposed by the Greek government. The rate of increase in acidity tended to rise with the temperature between these limits, and was greater during the early period of storage than after the first three months.

Durum wheat first clear flour had a lower initial acidity than the spring wheat second clear, ranging from 0.05 to 0.06%. It increased in acidity at a slower rate than the latter, reaching the limit of 0.15% in less than 150 days when stored at 25°C. and in proportionately shorter time at higher temperatures.

Patent our stored for more than five months still contained appreciably less than 0.15% of acidity.

Baking tests of the patent, first clear, and second clear flours, after five to seven months of storage, failed to reveal any evidence of unsoundness of the flour. This was true even after the clear flours contained more than the Greek limit of 0.15% of acidity.

The ratio of percentage of acidity, Greek method, to percentage of acidity, A.O.A.C. method, was not constant through the range studied, but tended to become narrower as the percentage of acidity increased.

Literature Cited

Bailey, C. H.
1925 The Chemistry of Wheat Flour. Chemical Catalog Co., New York.
Association of Official Agricultural Chemists
1925 Official and Tentative Methods of Analysis. Acidity of Water Extract—Tentative. p. 225.

BOOK REVIEW

Outlines of Biochemistry. The Organic Chemistry and the Physico-Chemical Reactions of Biologically Important Compounds and Systems. By Ross Aiken Gortner, Professor of Agricultural Biochemistry in the University of Minnesota, and Chief of the Division of Agricultural Biochemistry, University of Minnesota, and the Minnesota Agricultural Experiment Station. xv, 793 p. Illus. Fig. 133. John Wiley and Sons, Inc., New York. Chapman and Hall Ltd., London, 1929.

Dr. Gortner has written a readable and stimulating book for the use of an increasing number of students whose interests are not altogether those of the future doctor of medicine or research worker in physiology and pathology. Many notable textbooks on biological chemistry largely have been written with intended emphasis on the medical point of view. Such notable books on biochemistry as Abderhalden's "Lehrbuch," which was translated into English about twenty years ago, the last edition of which remains in the original German; the "Handbuch" of Oppenheimer, the English translation of Hammarsten, the Chemical Physiology of Halliburton, and the deservedly most popular American textbook of physiological chemistry by A. P. Matthews are of chief interest to students and workers in the field of medicine and allied research.

The fine work (may we not say classic) of the late W. M. Bayliss entitled "General Physiology" with its ingenious expositions of the relation of chemistry to the problems of physiology, also has a similar appeal but its influence has extended to many students in other fields.

The Outlines of Biochemistry, of Gortner, apparently has not been prepared with the needs of the medical student foremost in mind, but for the student whose interest brings him into contact with the biological problems of agriculture, agricultural products, the processes of food manufacture, and the properties of foods. The chemist engaged in the study of cereal problems, whether among those of the grain grower, the miller, or the baker, has long needed a general textbook on the rôles of organic and physical chemistry in biology for the better understanding and clarification of some of these difficult problems.

The subject matter of the book is broad in scope and includes the exposition of the following general subjects: The colloid state of matter, proteins, carbohydrates, and allied compounds, tannins, plant pigments, fats, lipides and essential oils, the vitamins and enzymes.

Recognizing the importance of colloid systems in the contemporary study of the living cell, Dr. Gortner devotes more than one third of his text to an exposition of colloids under ten chapter heads. These are compact with abstracts of researches, the comments of the author, and pertinent applications, many of which have a technical interest or implications.

Important and interesting are the discussions of the methods of preparing colloids, their physical properties, hydrogen-ion concentration, electro kinetic phenomena, surface and interfacial tension, surface energy and adsorption, electrolytes and colloid systems, a valuable chapter on gels, osmotic pressure, and some intelligible words on the Donnan equilibrium.

The reader will be impressed by noting that the author personally has been an active investigator of many of the subjects of which he has written and that his introduction to colloid study is not merely a compilation or digest of the existing literature.

Cereal chemists will find his discussion of viscosity and plasticity of special interest, mentioning as it does the well known work of Sharp and Gortner in its proper relation to the study of these properties of colloids.

The chapter on hydrogen-ion concentration also contains matter of interest to cereal chemists, including the well known observations of Gortner and Sharp on the influence of hydrogen-ion concentration on viscosity of flour-water suspensions. Dr. Gortner has included his criticism of the usual equation for calculating the dissociation constant of water, because of the probability that water is a mixture of water molecules of different degrees of polymerization, but he

tells us that since there is no means of determining the species and proportions of these molecules, it is impossible at present to test the hypotheses.

The author tackles the problem of the adsorption process with fervor and cites the famous "old clothes" illustration of A. P. Matthews and the "strawberry and snails" one of the late W. M. Bayliss, both of which are always good fun to read. However, the author thinks that probably both the physical and the chemical explanation of adsorption are correct, depending upon conditions for the predominance of one over the other. Primary valence is not excluded, except in certain cases, and the author apparently agrees with H. S. Taylor in his conception of active surface valences. The discussion of adsorption is carried on with much interest in reference to catalysis and the technical applications of adsorption.

The chapter on electrolytes and colloid systems is a valuable summary of observations and current ideas on flocculation by electrolytes and the subject of protective colloids. Considerable attention is given to the problem of the Hofmeister or lyophilic series, in which the work of D. R. Briggs is cited at length and commented upon by the author, who states that "Briggs is undoubtedly correct in arguing that the behavior of any chemical ion will depend not only upon the concentration of that ion but also upon the nature of the system, and upon the type of reaction which is being studied." This conception may explain the differences in the behavior of various lyotropic series in the hands of different workers.

The chapter on gels is good reading. The discussion of imbibition and the rôle of water in living organisms brings up many important applications in biology and agriculture. Cereal chemists will find their chief interest in the author's treatment of imbibition in which the first work on the quality of wheat flour proteins by Upson and Calvin, and Gortner and Doherty is mentioned, and the subsequent studies of Oswald and Luers, Gortner and Sharp, and Luers and Schneider.

The importance of osmotic pressure in living processes is emphasized in chapter nine, and here reference is made to the well known work of J. A. Harris, with whom the author has been associated in important studies on the concentration of tissue fluids of plants of arid regions. The concluding chapter of the colloid section of the book is devoted to a consideration of the "Donnan equilibrium." This is pointed by reference to some of the puzzles of membrane permeability and selective adsorption in plant physiology.

Section II of the book contains nine chapters on the proteins and one on the nitrogen bases. Here is the foundation for another textbook. Recent researches on the theories of protein structure and isomerism, properties of proteins and their biological relations are presented. The students of the chemistry of cereals will find this section of much value for its introduction to some of the contemporary ideas about proteins which have not yet filtered into the usual textbooks.

Dr. Gortner dwells at some length on the acid and base binding capacities of proteins, a subject to which he has made contributions of both data and theories. In discussing the work of E. J. Cohn, new unpublished data are introduced in support of Gortner's contention that the amount of base bound to casein is dependent upon the equilibrium hydrogen-ion concentration.

This statement obviously does not agree with Cohn's ideas about the maximum base binding capacity of casein. The interesting charts of C. Mez, on the relationship of plant species and genera as indicated by serum diagnosis will give the student a graphic picture of the relation of protein to the plant family tree.

The third section on the carbohydrates presents contemporary ideas on their synthesis in nature, classification, chemical structure, and properties. Some attention is given to various types of fermentation; and the changes that sugars undergo in plants also are emphasized. The discussion of the polysaccharides contains material of comparatively recent publication, among which is mention of the little known bacterial polysaccharides which precipitate antisera and which seems to indicate that polysaccharides as well as proteins have their relation to the immunity problem. The new and important work of Sponsler and Dore on the structure of cellulose obtained from the study of X ray data receives consider-

able mention and is well illustrated by reproduction of the graphs worked out by these investigators. The glucosides and their properties are described and tabulated with special attention to structure, and synthesis. The chapter on the pectic substances, or pectins, was contributed by J. J. Willaman, formerly of the University of Minnesota, but now chief of the Division of Chemistry at the New York (Geneva) Experiment Station. This is a clear, concise summary of the pectin problem. Constitution, properties, and biological significance are discussed, with some reference to commercial pectins.

The tannins and their biological relations receive concise treatment and the problems of the plant pigments are presented in Section V. Considerable attention has been given to the inheritance problems of color, a subject in which the author has had an early and intimate chemical interest.

The fats, lipides, and essential oils are presented in the four chapters of Section VI. In addition to fundamentals and classification, the section contains a discussion of rancidity in the chapter on fats and oils. This might have been strengthened by reference to the work of W. C. Powick.

The sterols receive considerable attention, as these compounds have recently come into prominence because of the recognition by investigators that when properly irradiated with ultraviolet light ergosterol has similar properties to vitamin D.

The two final chapters of the book are on the vitamins and enzymes, respectively. The chapter on vitamins has been written by L. S. Palmer, of the University of Minnesota, an able investigator and contributor to this field of biochemistry. Dr. Palmer's contribution is another textbook in little, and his survey is a most valuable one not only for beginners but for more experienced students. The chapter on enzymes by the author concludes the book. It presents the catalytic viewpoint of enzyme reactions, and the author is apparently in harmony with the general ideas of the Willstätter School on the nature of enzymes. The influence of hydrogen-ion concentration, time, and temperature, on enzyme reactions receives much emphasis from the author's style and the beginner in enzyme study receives definite warning as to pitfalls.

This review of Gortner's Outlines of Biochemistry has been necessarily brief and it cannot do justice to the scope and value of this book. The reading of it has convinced the reviewer again that dullness need not be inevitable in a scientific textbook, and tho the personality of the author sometimes has not been subdued by the difficulties of the subject or the desire to hide his own opinions under a mass of quotations, this should not work against the success of the book or its future influence.

The typography is good, the proof appears to have been carefully read, and misprints are few. The citations to the literature by page footnotes are many. They appear to have been well checked. The general references include most of the authoritative works pertinent to the scope of this book, and publications of 1928 are noted. The author and subject indexes are adequate.

—C. Brewster Morison.

INDEX—AUTHOR AND SUBJECT

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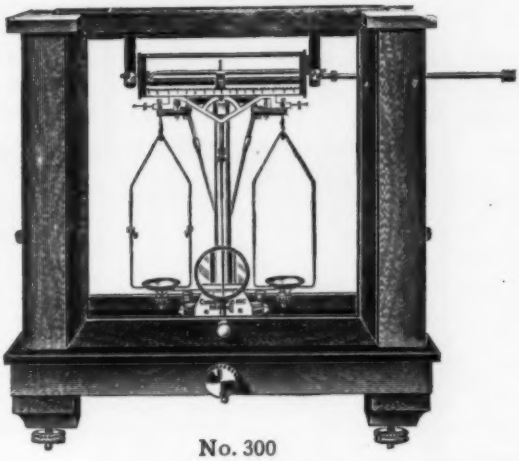

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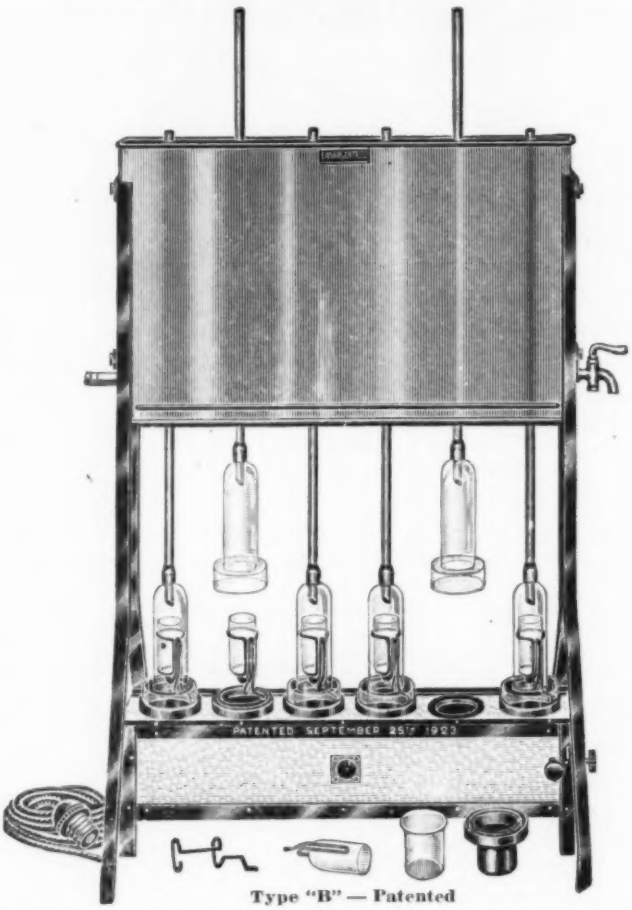

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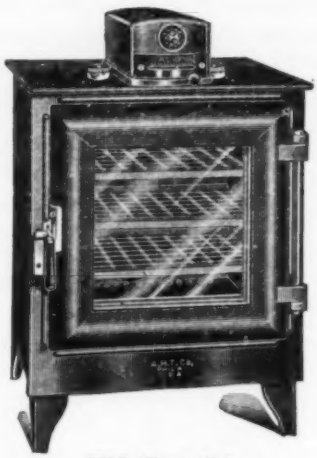

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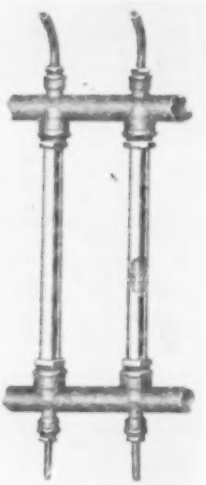

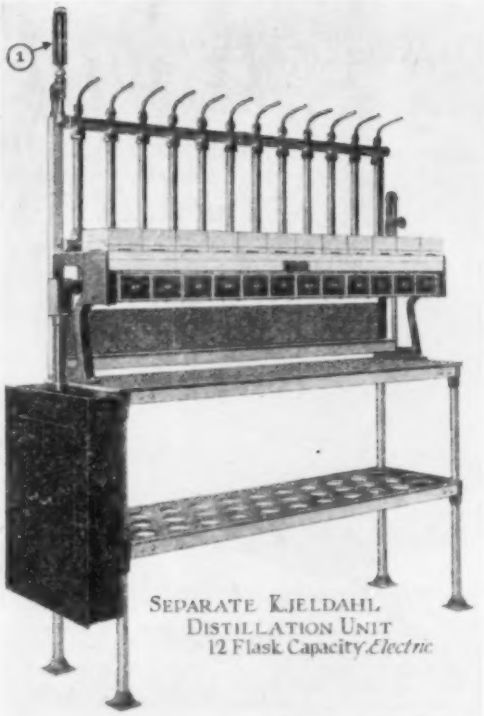

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